ECETOC

MONOGRAPH No. 7

BRUSSELS, DECEMBER 1986

MONOGRAPH No. 7

RECOMMENDATIONS FOR THE HARMONISATION OF INTERNATIONAL GUIDELINES FOR TOXICITY STUDIES

CONTENTS

The reader should note that the text is organised as follows. The problems dealt with in the report, and the purpose and scope of the document, are described in chapter A. Readers looking for the key issues to be taken into account in considering harmonisation will be most interested in Chapter A, Chapter C in which the more important differences between the OECD and the other main guidelines are set out, and Chapter D in which recommendations are made for harmonisation of the guidelines.

The detailed account of the various guidelines in Chapter B, and the extensive tabulation of the differences between them in the Appendices, will be of interest primarily to practicing toxicologists.

FOREWORD

Over the past few years the European Chemical Industry Ecology and Toxicology Centre (ECETOC) has published a number of Monographs in which it has attempted to clarify, and express its views on, some of the more important problems in toxicology. This Monograph is a further addition to the series.

The harmonisation of international guidelines for toxicity studies on chemicals, while certainly requiring discussions of some quite specialised scientific matters, has broader implications for society as a whole. Skilled toxicologists are a scarce resource with respect to the extent of the problems which they have to deal with. Too often during the gathering of experimental data on chemicals to be notified to (or registered with) the authorities, studies already done for one country have to be repeated to satisfy the requirements of another. This repetition usually has to be made because of differences in the requirements between countries, the differences being rather minor in some cases, and often not scientifically justified. Hence our scarce toxicological resources are wasted. Furthermore, these wasted resources could have been used to gain new knowledge of the toxic effects of other chemicals, to the benefit of all.

This inefficient use of resources has also the undesirable and, in fact, immoral feature that in order to assess the toxicological profile of a chemical more experimental animals are used than is necessary. Furthermore, there is usually no gain whatsoever in our toxicological knowledge resulting from the repetitious studies performed.

I therefore recommend this Monograph to all who are concerned with the most effective use of our resources in toxicology in the broadest sense, and trust that moves towards harmonisation can be taken in the near future.

Dr. H.J. Heller
Chairman of ECETOC Board
Director of Ciba-Geigy

SUMMARY

Several organisations have issued guidelines or requirements for toxicological studies on industrial chemicals (OECD, EEC, UK/HSC and EPA/TSCA) and pesticides (EPA/FIFRA and Japan/MAFF) over the past few years - see Appendix 1 for abbreviations. Differences between these various documents can lead to the duplication of studies, the unnecessary use of animals and unjustified expenditure, when a substance has to be notified or registered worldwide. Harmonisation of the guidelines and requirements, without the loss of essential flexibility, would therefore be highly desirable.

In this Monograph the requirements for subchronic (14-90 days), chronic, carcinogenicity, combined chronic and carcinogenicity, and reproductive toxicity studies on industrial chemicals and pesticides in the above-mentioned guidelines are compared in detail with the aim of identifying the main differences. For this comparison, the OECD "Test Guidelines" are taken as yardsticks because they are the most widely-recognised internationally. Recommendations are made for harmonisation, in some cases comprising suggestions for up-dating the OECD Test Guidelines by adopting some of the better features from the other documents, where there is no scientific justification for the differences. The recommendations fall into two groups : those for harmonising general requirements which are common to all of the studies and those for harmonising important details within each individual type of study.

A. INTRODUCTION, PURPOSE AND SCOPE

A number of organisations have issued Guidelines for toxicity studies or Directives for the toxicological assessment of chemicals and pesticides. The methods described in these are generally quite similar but there are often differences in detail. The OECD has taken a major initiative in attempting to harmonise toxicity studies by the development and publication of a series of Test Guidelines (TGs) in 1981, 1983, and 1984. Most of the countries with significant regulatory requirements have adopted these guidelines for the toxicological assessments of chemicals (OECD, 1981). There remain, however, some differences in practice between national requirements and the OECD TGs. With respect to worldwide notification or registration this leads to the repetition of studies, which is often not scientifically necessary, and may call for the unreasonable use of animals and unnecessary expenditure. It would be advisable therefore to harmonise these TGs as far as possible. This should be done without restricting flexibility so that toxicologists can design the most suitable protocol for each study carried out.

To examine this problem, ECETOC therefore set up a Task Force (TF) with the following Terms of Reference :

1. To collect and list the existing or proposed regulatory guidelines for toxicological studies on industrial chemicals and pesticides from the OECD, the European Communities (EEC), UK, USA, and Japan. Consideration is to be limited to subchronic and chronic toxicity, carcinogenicity, combined chronic toxicity/carcinogenicity and reproductive toxicity (including teratogenicity) studies.

2. To identify the differences in the above guidelines and to assess whether they are practically and scientifically justified.

3. To recommend on the basis of the above assessment :
   a) up-dating of the appropriate OECD Test Guidelines;
   b) modifications to any of the guidelines which would lead to greater international harmonisation.

   The Task Force has reviewed the TGs for industrial chemicals from the OECD (1981), the EEC (1983, 1984), the UK/HSC (1982) and the EPA/TSCA (1982,

1983-1984); for abbreviations, see Appendix 1. The Scandinavian countries and Canada have issued some general guidance for subacute and chronic studies, but because detailed descriptions were lacking these were not included in the comparison.

The existing or proposed regulatory guidelines for toxicological studies on pesticides from the EPA/FIFRA (1985, draft) and Japan/MAFF (1985) were also considered since these guidelines have served as a basis of TGs for industrial chemicals. They are tabulated and compared in the text and Appendices in parallel with the TGs for industrial chemicals. This procedure was chosen in order to emphasise the differences. The Task Force notes that in a few cases the differences between the Japan/MAFF TGs and others could be due to difficulties in translation into English as it was not possible to consult the original Japanese text.

The following procedure has been used in this Monograph to bring out the differences between the TGs and to arrive at the recommendations. Firstly, the major features of each specific study type (i.e., subchronic, chronic, etc.) have been described in Chapter B and compared in the Appendices of Chapter E. These have been summarised in the text of Chapter C and in each case the differences between each TG and OECD (used as the yardstick) have been highlighted. Finally, in Chapter D, the Task Force has made recommendations for further harmonisation of the TGs. The original text of the TGs was reflected as far as possible in the Table Appendices to emphasise any differences.

It is emphasised that some requirements are not mentioned in the TGs since they are covered by the principles of Good Laboratory Practice (GLP; OECD, 1981-a), especially as Standard Operating Procedures. Aspects of study planning and reporting requirements are also considered to be subject to GLP.

B. DESCRIPTION OF CURRENT TEST GUIDELINES

1. INTRODUCTION

It is recognised in many guidelines that the types of study to be carried out depend to a great extent on the properties of the chemical, while the conduct of such studies relies upon the expertise of those involved in their design

and supervision. The design of experiments should be planned carefully by close cooperation among all experts involved.

A critical comparison of the various TGs is hampered by several factors. Some TGs are simply recommendations while others are requirements. Furthermore, the study of different types of chemicals (pharmaceuticals, food additives, pesticides, industrial chemicals, etc.) is based on different historical backgrounds so that the structure, organisation and wording of TGs is not uniform. In some TGs certain criteria and requirements are described explicitly, whereas in others they are dealt with only in general terms or are not mentioned at all. The OECD TGs systematically list and define their purpose, scope, relevance, application and limits in the methodology sections. In other TGs, these may be divided between the introduction, the general part of the text or the list of study observations. The Task Force has shown some of these differences in the Appendices. In these a clear distinction is intended between the phrases "not mentioned" and "not specified" (the latter meaning: mentioned but without specifying details) and the Task Force has used its own interpretation of the text, instead of word by word transcription, where it was evident that the meaning is the same.

The Task Force recognised that some of the principles in the TGs were established on a practical rather than a purely scientific basis. It also realised that topics such as test substance, caging, diet and water supply, etc. could be treated as being common components of all TGs. Other topics, such as test substance identification, were considered to be part of GLP rather than of a TG..

2. CRITERIA, REQUIREMENTS AND STUDY OBSERVATIONS COMMON TO VARIOUS TYPES OF TOXICITY STUDIES

Those general requirements and study observations which are common to all guidelines are discussed in this section. Some aspects specific to reproductive toxicity are dealt with in section B.7. All other criteria have been collated in tables and are discussed in the sections dealing with specific TGs.

2.1. Animals

a) Age at start of exposure. Most TGs recommend that oral treatment starts before the animals are mature. For rodents, dosing preferably

starts soon after weaning, ideally before 6 weeks, but in any case not later than 8 weeks, of age. There should be an acclimatisation period of at least five days before a study starts. The preferred age for dogs is 4-6 months and not more than 9 months. The age for other non-rodent species is not specified. In subchronic inhalation studies the age at the start of exposure is not well-defined, while in subchronic dermal studies the body weight rather than the age of animals suitable for use is given.

Most TGs state that female animals should be nulliparous and should not be pregnant.

b) Caging. Recommendations on housing conditions appear somewhere in all TGs. In the OECD TGs they are detailed, while all others mention caging in the preamble (EEC, EPA/FIFRA, UK/HSC) and/or only in relation to specific parts of the TGs (e.g. inhalation or dermal toxicity studies). In general, caging should be adequate for the species. Where groups of animals are caged together, the number per cage should not interfere with the clear observation of each individual (OECD). For oral and inhalation studies the animals may be caged in groups, males and females separately, or individually. In dermal studies, animals should be caged individually (not mentioned by Japan/MAFF).

c) Environmental conditions. Details about temperature, relative humidity and light regime are given in the OECD TGs and in the preamble to the EEC and UK/HSC TGs (rodents only). The EPA/FIFRA guidelines provide general information on environmental conditions in the preamble. With the exception of Japan/MAFF, all authorities make some recommendations about the environmental conditions for inhalation studies, with details of temperature and relative humidity.

d) Diet and water. Precise guidance concerning diet and water is not given because of differences in supply and in national requirements. The OECD details the dietary requirements in most of its TGs. The EEC and UK/HSC give some information in their preambles while EPA/TSCA and Japan/MAFF give none and EPA/FIFRA provide only indications in the general provisions of their guidelines.

2.2. Treatment

a) Requirements for test compound. Although these requirements are not referred to in all guidelines it is expected that the test compound will be characterised and its analytical purity determined. Toxicity studies should usually be performed with "technical grade product or with the technical grade of active ingredients in a product" (OECD).

b) Route of administration. The exposure conditions should correspond or be relevant to those in man, but the choice of route also depends upon the physical and chemical characteristics of the test substance. In oral administration, it may be given in the diet, the drinking water, by gavage or in capsules.

Some of the TGs describe the requirements for inhalation experiments in more detail, and the provisions for subchronic and teratogenicity testing are given in a specific TG - see Appendices 2 (cont. 6 and 7) and 7 (cont. 6 to 8). For chronic, carcinogenicity and combined studies, specific provisions for inhalation exposure are part of the general TGs (Appendix 6).

c) Frequency of dosing. Ideally the animals should be treated 7 days per week, but for practical reasons 5 days per week is acceptable. Specific regimes more relevant to human exposure are sometimes described.

2.3. Study Observations - Clinical Data

a) Body weight. This should be measured regularly throughout all toxicity studies, weekly up to 13 weeks and then once every 4 weeks.

b) Food consumption. Where measurement of food consumption is required it is carried out weekly during the first 13 weeks of the study period. Thereafter, measurements are made at monthly (EPA,TSCA and FIFRA; Japan/MAFF) or three-monthly (OECD, EEC, UK/HSC) intervals. In addition, according to Japan/MAFF, food efficiency should be calculated during the growth period of the animals.

c) Water consumption. When the test substance is administered in the drinking water, the water consumption should be measured at the same intervals as is food consumption (not mentioned in all TGs).

2.4. Reporting

The study report must include all information necessary to provide a complete and accurate description of the procedures and an evaluation of the results. It should contain a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight data or observations which may indicate toxic effects.

3. SUBCHRONIC TOXICITY STUDIES

3.1. Introduction

Subchronic toxicity studies in animals are designed to investigate possible adverse effects occurring as a result of repeated dosing of a chemical in graduated doses to several groups of experimental animals for part of their lifespan not exceeding 10%. Well-designed subchronic studies lead to the identification of the target organs and provide information on accumulation potential, no-effect levels and maximum tolerated dose levels. The results of these studies are used for the evaluation of risk to human health and the environment. They also provide information for selecting dose levels for subsequent toxicity studies of longer duration.

In this Monograph all studies of duration between 14 and 90 days are covered under the heading of subchronic toxicity studies. These include those studies traditionally referred to as subacute, short-term, repeated-dose studies, etc.

OECD, EEC and UK/HSC have issued TGs for 28-day studies for oral and inhalation exposure (OECD 14/28 days), and for dermal treatment (OECD 21/28 days). EPA/FIFRA and Japan/MAFF have issued TGs for 21-day dermal studies. All regulatory bodies have issued TGs for 90-day studies with oral treatment, inhalation exposure and, except Japan/MAFF, dermal treatment.

The TGs describing subchronic studies on neurotoxicological effects are not within the scope of this Monograph.

3.2. Discussion of the Guidelines

3.2.1. Animal criteria

a) Species. For oral exposure, TGs exist for one rodent and one non-rodent mammalian species. The rat is the preferred rodent species although some TGs (OECD; EPA, TSCA and FIFRA) allow a variety of such species and other TGs (EEC and UK/HSC) accept other rodent species if "contra-indications" against the rat exist. The dog is the preferred non-rodent species, although other species, for the selection of which justification/reasoning has to be given, may be used (all TGs except Japan/MAFF). Japan/MAFF requires studies with at least two mammalian species, a rodent and a non-rodent. No preferred species or strain is, however, indicated.

The preferred species for dermal exposure in all TGs are the rat, rabbit or guinea pig. Other species are allowed provided that justification for their selection is given.

The OECD issued TGs for inhalation exposure only for rodents (rats preferred). Japan/MAFF requires at least one mammalian species, but the rat is preferred. In the EEC and UK/HSC TGs the rat is preferred unless "contra-indications" exist. EPA (TSCA and FIFRA) allow a variety of rodent species, but the rat is preferred. The last two guidelines require justification/reasoning to be given if another mammalian species is used.

When a subchronic study is used as a range-finding study for long-term treatment, the same species and strain has to be used in each (Japan/MAFF).

b) Group size. The number of animals per group should be sufficient to allow a proper statistical evaluation of the results obtained in order to reveal toxicologically important effects. For rodents, at least 5 males and 5 females per group are required for 14/21/28-day studies and at least 10 males and 10 females for 90-day studies. The minimum group size for non-rodents is 4 males and 4 females in 90-day studies.

The number of animals must be increased if interim sacrifices are planned. In addition, satellite groups of the same number of animals

treated at the high dose level may be used to investigate the reversibility, persistence, or delayed occurrence of toxic effects for a certain recovery period (14 or 28 days, depending on the duration of the treatment) following treatment. However, when such a satellite group is used it is not mentioned in the TGs whether a concurrent control group is also needed.

3.2.2. Treatment

a) Dose level/No. of control groups. At least three dose levels and a concurrent control group are required :

i) a low dose level which should not produce evidence of toxicity but should exceed the expected human exposure level (not specified by Japan/MAFF);

ii) an intermediate dose level or levels which should produce only minimal toxic effects. If more than one intermediate dose is used the dose levels should be spaced to produce a gradation of toxic effects (not specified by Japan/MAFF);

iii) a high dose level which should produce toxic effects and indicate target organs. It should not produce an incidence of fatalities in rodents which would prevent meaningful evaluation, and should produce no fatalities in non-rodents.

In the case of a test substance with low toxicity, a "limit test" with only one (high) dose level, and a concurrent control group, may be adequate (not mentioned by Japan/MAFF).

b) Duration and frequency of treatment. Depending on the purpose of the study, oral treatment in rodents is usually for at least 14 or 28 days and/or 90 days, and in non-rodents at least 90 days. Inhalation exposure is usually for at least 6 hours per day for 14 or 28 days and/or 90 days. Dermal treatment is usually for at least 6 hours per day for 21 or 28 days and/or 90 days. In general, the animals are treated 7 days/week, but for practical reasons 5 days/week is considered acceptable.

3.2.3. Study observations

a) Clinical data

i) Clinical signs and mortality. Observations for signs of toxicity, including time of onset, degree and duration, should be made at least once per day. In addition, observations of dead or moribund animals should be made daily. Moribund animals should be sacrificed in order to prevent cannibalism and/or autolysis.

ii) Ophthalmology. Examination of the external and internal eye is required in 90-day studies in rodents and non-rodents, initially in all animals of the control and high dose groups (Japan/MAFF: preferably in all animals of the study but at least in high dose and control groups), prior to the start and at the end of the treatment period. If changes are detected, all dose groups should be examined. EPA/TSCA requires that 5 males and 5 females of all rodent groups, and all animals of all non-rodent groups, should be examined.

b) Haematology, clinical chemistry and urinalysis. Haematology and clinical chemistry determinations should be performed on all rodents at the end of the study. EPA/TSCA requires, in addition, an investigation shortly before the start of treatment, and after 30 days of treatment, on 5 males and 5 females of all groups.
Haematology and clinical chemistry on all non-rodents is required prior to the start, at monthly intervals during, or midway through, the dosing period (for EPA/TSCA after 30 days of dosing) and at the end of this period. A limited range of clinical chemistry parameters is required by EEC and UK/HSC in the 14- or 28-day studies. Urinalysis is not required on a routine basis.

c) Post-mortem examinations

i) Gross pathology. All animals found dead or sacrificed in a moribund state during the study period, or sacrificed at the end of this period, should be subjected to a full gross necropsy.

ii) Organ weights. At least a minimum set of organs (e.g.kidneys, liver and testes in all TGs) from all animals in the study, including those which died or were killed in a moribound state during the study, should be weighed.

iii) Histopathology. A range of organs (similar but not exactly

identical in the different guidelines) should be examined microscopically in rodents, initially in all animals of the control and high dose groups. The same is true for non-rodents, but EPA (TSCA and FIFRA) and Japan/MAFF require histopathology in all animals. The UK/HSC and EEC guidelines for the 14- or 28-day studies require examination of a limited range of organs. In addition, for rodents, organs showing gross lesions, target organs, kidneys, liver and lungs should also be examined microscopically in the lower dose groups.

4. CHRONIC TOXICITY STUDIES

4.1. Introduction

The aim of a chronic toxicity study is to administer the test compound for a long enough period, at least one year, for chronic effects to be realised. The number of animals and dose levels should be sufficient to establish dose-response characteristics and a no-effect level. The range of examinations within the study should be sufficient to cover several parameters of toxicity and, where possible, the examinations should be undertaken at intervals throughout the study to follow the development of chronic effects.

Although the situation of chronic toxicity studies looks clear when viewed on its own it can become confused in practice because its assessment may take place during the conduct of long-term rodent studies for carcinogenicity. It has become the practice in these circumstances to incorporate satellite groups for sacrifice at one year to accomplish the chronic toxicity element of the study. The combined chronic toxicity/carcinogenicity studies are considered in more detail in section 6 of this chapter.

4.2. Discussion of Guidelines

There is a reasonable agreement on the requirements for chronic toxicological evaluation in the various TGs. However, there are some differences in detail as discussed below and shown in Appendix 3. It should be noted that the UK/HSC mention the possibility of a long-term toxicity study as "an additional test method" but do not have a guideline for such a study.

4.2.1. Animal criteria

a) Species. All TGs advocate that the rat be used. Some authorities require chronic toxicity studies to be undertaken in two species. The OECD and EEC leave the issue of a second species rather vague, inferring that its use depends upon circumstances and that in some cases it may not be necessary. The EPA (TSCA and FIFRA) and Japan/MAFF require a non-rodent, preferably the dog, as second species.

b) Group size. At least 20 male and 20 female rodents, or at least 4 male and 4 female dogs, are required for each group. If interim sacrifices are planned, the number is increased by the number of animals scheduled to be sacrificed before the completion of the study.

4.2.2. Treatment

a) Dose levels/No. of controls. There should be at least three dose levels to establish dose-response characteristics and a no-effect level. One concurrent control group is sufficient. The high dose should produce evidence of a definite effect and the low dose should have no effect. The role of the intermediate dose varies between guidelines, the EPA (TSCA and FIFRA) requiring some minimal evidence for an effect. The OECD, EPA (TSCA and FIFRA) and Japan/MAFF recommend an upper dosing limit of 5 % of test compound in the diet.

b) Route and frequency of administration. The route should be appropriate to that expected for exposure of man. Dosing by the oral route (dietary, etc.) should be for 7 days/week although most authorities allow a dispensation for 5 days/week in special circumstances. The execution of inhalation studies is dealt with in detail by the OECD and EEC, and in their TGs a regime of 6 hours/day for 5 days/week is acceptable. There is very little guidance on the conduct of dermal application studies.

c) Duration of Treatment. Twelve months is the minimum agreed period for dosing in rodent or non-rodent studies except for Japan/MAFF who require rodent studies to continue for 18 months (mice) or two years (rats).

4.2.3. Study observations

a) Clinical data

i) Clinical signs and mortality. Observations to be made daily.

ii) Ophthalmology. Specified only in the EPA (TSCA and FIFRA) and Japan/MAFF requirements.

b) Haematology, clinical chemistry and urinalysis. Assays are undertaken at specified regular intervals during the studies, e.g. at 3 and 6 months, and then every 6 months for haematology and urinalysis and at every 6 months for clinical chemistry. All non-rodents, and at least 10 rodents per group, should be examined. There are some differences between guidelines concerning the number of parameters investigated but the aspects covered are the same.

c) Post-mortem examinations

i) Gross pathology. All animals should be examined post mortem.

ii) Organ weights. A selection of organs should be weighed. There are detailed differences between guidelines but liver, kidneys, brain and testes are included in all instances.

iii) Histopathology. Microscopic examination should be undertaken on a range (approximately 30) of tissues in all rodents and, as far as the OECD and EEC TGs are concerned, on all non-rodents dying or sacrificed during a study as well as on those animals terminated at the end of the study in the control and high dose groups. Intermediate dose levels are to be examined if deemed necessary by the pathologists/ toxicologists. The EPA (TSCA and FIFRA) and Japan/MAFF require all non-rodents from the study to be examined.

5. CARCINOGENICITY STUDIES

5.1. Introduction

The aim of a long-term carcinogenicity study is to observe experimental animals for the development of neoplastic lesions during or after exposure for a major portion of their life to various doses of a test substance. Most recent TGs, including that of the OECD, are based on an IARC publication containing a critical discussion of the assessment of carcinogenicity (IARC, 1980). Discussions of chemical carcinogenesis studies and the evaluation of results are still going on within the

scientific community: see for example the activities of the ad hoc Panel on Carcinogenesis Testing of the US National Toxicology Programme (NTP, 1984).

5.2. Discussion of Guidelines

5.2.1. Animal criteria

a) Species. For a compound of unknown activity, assays with two animal species are recommended, rats and mice being preferred. Syrian hamsters are mentioned as particularly suitable for studies on respiratory tract carcinogenesis. Non-rodent species, primates, or dogs are mentioned (OECD, EEC), but are not further considered in the TGs reviewed. If it is considered necessary to carry out a study with species other than rats or mice it is recommended that this should be discussed with the authorities in advance. This also is recommended if exposure regimes deviating from the normal (e.g. exposure of prenatal or neonatal animals) are considered. The choice of the animal strain is debated in some of the TGs. Commonly-used laboratory strains whose tumour profiles are well-known are generally preferred.

b) Group size. At least 50 male and 50 female rodents are required for each dose level and for the control(s). If interim sacrifices are planned, the number should be increased accordingly.

5.2.2. Treatment

a) Dose levels. Three dose levels are recommended so that information on the dose-response relationship can be obtained.

i) The low dose should not interfere with the normal growth, development and longevity of the animals, or otherwise give any indication of toxicity. In general, the low dose should not be less than 10% of the high dose (OECD, EEC, UK/HSC, EPA/TSCA).

ii) The intermediate dose should be in the mid-range between the high and the low dose. OECD and EPA/TSCA mention the use of toxicokinetic properties of the chemical, if known, in setting the intermediate dose.

iii) The high dose should be sufficiently high to elicit signs of minimal toxicity (e.g. a depression of body-weight gain of less than 10%) without substantial alteration in the normal life-span due to effects other than tumours (except EPA, TSCA and FIFRA).

b) Duration of treatment. This depends on the normal life-span of the strain of test animal. In general, for mice the test is terminated at 18 months, and for rats at 24 months. With certain strains of mice or rats of greater longevity or low spontaneous-tumour rate the termination may be extended to 24 or 30 months, respectively. Alternatively, termination of the study is acceptable when the number of survivors in the lowest dose or control group falls to 25%. When there is an apparent sex difference in response, the results from each sex should be considered separately.

"For a negative test to be acceptable"(OECD, EEC, UK/HSC) not more than 10% of any group may be lost because of autolysis, cannibalism or management reasons, and survival of all groups should be at least 50% at 18 and 24 months in the case of mice and rats, respectively.

5.2.3. Study observations

a) Clinical data. The animals are observed daily by physical examination. Special attention must be paid to tumour development. The time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumour should be recorded. Detailed clinical examination of sick animals is necessary to provide a diagnosis.

b) Haematology. At 12 and 18 months and at sacrifice a blood smear is obtained from 10 animals/sex/group (EPA/FIFRA) or from all of the animals (all other TGs). A differential blood cell count is performed on samples of the animals in the highest dose group and the controls. If necessary, it is performed on samples of the next lower dose group as well and also on sick animals.

c) Post-mortem examinations. Complete gross pathology should be carried out on all animals. All visible tumours, and lesions suspected of being tumours, should be preserved. Organs and tissues of all animals should be preserved for microscopic examination. Organ weight determinations are requested by EPA/FIFRA and Japan/MAFF.

Histopathology should be performed :

i) on all grossly visible tumours, or lesions suspected of being

tumours, in all groups;

ii) on all preserved organs and tissues of the animals that died or were killed during the study;

iii) on all preserved organs and tissues of the highest dose group and controls;

iv) on the organs or tissues of all animals in the study in which significant differences in hyperplastic, pre-neoplastic or neoplastic lesions are observed between the highest dose and control groups;

v) if excessive early deaths or other substantial alterations occur in the highest dose group, in which case the group at the next lower dose level should be examined as described for the highest dose group.

6. COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDIES

6.1. Introduction

The objective of a combined chronic toxicity/carcinogenicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. The design and conduct of the study, according to the OECD, EEC, Japan/MAFF, and EPA (TSCA and FIFRA) guidelines, should allow neoplastic effects to be detected and carcinogenic potential and general chronic toxicity to be determined. There is no UK/HSC guideline for such a study.

6.2. Discussion of Guidelines.

The guidelines have common general requirements, but there are numerous differences in detail.

6.2.1. Animal criteria - Species. Generally only one species, preferably the rat, is required for a combined chronic toxicity/carcinogenicity study. If other species (e.g. dog, mouse, hamster) are used, most guidelines request a reason for their selection. For a valid assessment, the strain selected should be susceptible to the carcinogenic or toxic effect of the class of substance being studied and should not have too high a spontaneous-tumour background. Commonly-used laboratory strains should be employed. If interim sacrifices are planned, the number of animals should be increased by the number scheduled for sacrifice.

6.2.2. Treatment

6.2.2.1. Special requirements for the carcinogenicity part

a) Dose levels, group size. In assessing carcinogenicity, at least three dose levels should be used in addition to the concurrent control group. The selection of these dose levels is usually based on existing data, preferably on the results of a subchronic study. There should be at least 50 males and 50 females in these groups. The dose levels are as follows :

i) The lowest dose level should produce no evidence of toxicity. In general, it should not be less than 10% of the high dose (OECD, EEC).

ii) The intermediate dose level(s) should "produce minimal observable toxic effects" (EPA, TSCA and FIFRA) or "be established in a mid-range between the high and low doses" (OECD, EEC).

iii) The highest dose level should elicit "signs of minimal toxicity" (OECD, EEC, Japan/MAFF) or "signs of toxicity" (EPA, TSCA and FIFRA) without substantial alteration of the normal life-span due to effects other than tumours.

"For a negative test to be acceptable"(OECD, EEC, UK/HSC) not more than 10% of any group may be lost because of autolysis, cannibalism or management reasons, and survival of all groups should be at least 50% at 18 and 24 months in the case of mice and rats, respectively.

b) Duration of study. According to all guidelines (except Japan/MAFF) the termination of the carcinogenicity part of the study should be at 18 months for mice and hamsters and 24 months for rats. However, with certain strains of animals of greater longevity and/or low spontaneous tumour rate, termination should be at 24 months for mice and hamsters and 30 months for rats. Alternatively, the OECD and EEC guidelines permit termination of the study if the number of survivors in the low or middle dose, or control, group falls to 25%. If there is an apparent sex difference in response, the studies on each sex should be terminated separately. The Japan/MAFF guidelines do not specify exactly how long this part of the study should last, but suggest that the duration encompasses most of the normal life-span.

6.2.2.2. Special requirements for the chronic toxicity part

a) Dose levels/satellite groups. For the assessment of chronic toxicity, the carcinogenicity study is supplemented with one or more treated satellite groups and a control satellite group. The various regulatory authorities require a different number of animals for these groups. The highest dose for satellite animals should produce "frank toxicity" (OECD; EPA, TSCA and FIFRA and Japan/MAFF) or "definite signs of toxicity" (EEC) in order to elucidate a toxicological profile of the test substance.

b) Duration of study. In general, the satellite dose group(s) and the concurrent satellite control group kept for evaluation of chronic toxicity should be retained for at least 12 months. According to EPA/FIFRA the satellite groups should be dosed for 12 months in the case of chemicals not used in food, and at least 24 months for chemicals which are used in food, while Japan/MAFF suggests a dosing period for the satellite animals of 24 months (rat) or 18 months (mouse). These animals should be scheduled for sacrifice such that an estimate of test substance-related pathology, uncomplicated by geriatric changes, can be made.

6.2.3. Study observations.

a) Clinical data. Clinical examinations should be made at least once per day. Additional observations should be made daily, with appropriate action to minimise the loss of animals. Special attention must be paid to tumour development. The time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumour, and the time of onset and progression of toxic conditions, should be recorded.

Only the two EPA guidelines and those from Japan/MAFF require ophthalmology prior to administration of the test substance and at termination of the study.

b) Haematology, clinical chemistry and urinalysis. Haematological examinations should be performed on blood samples collected from 20 (OECD, EPA/TSCA) or 10 (EEC, EPA/FIFRA, Japan/MAFF) rats/sex of all

groups. If possible, these samples should be taken from the same animals at each interval.

Blood sampling should take place at 3 months, 6 months, at approximately 6-month intervals thereafter and at termination (OECD, EEC, EPA/TSCA), or at approximately 6-month intervals and at termination (EPA/FIFRA, Japan/MAFF). If haematological effects are found in the subchronic study, Japan/MAFF requires an additional examination after 3 months.

The TGs, with the exception of Japan/MAFF, require differential blood-cell counts in animals of the control and highest dose groups. In addition, if there is a major discrepancy between the highest dose group and the controls, these investigations should be performed also for the next lower dose group(s). If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals should be performed.

Individually-specified determinations of clinical chemistry on blood should be carried out at approximately 6-month intervals and at termination (OECD, EEC, EPA/FIFRA, Japan/MAFF), or just prior to the initiation of dosing, near the middle, and at the end of the test period (EPA/TSCA). Blood samples for clinical chemistry measurements should be drawn from at least 10 rats/sex of all groups and, if possible, from the same rodents at each time interval. Determinations of electrolyte balance, carbohydrate metabolism, and liver and kidney function are considered appropriate to all studies. The selection of specific tests should be influenced by any available observations on the mode of action of the substance.

Urine samples should be collected for analysis from 10 rats/sex of all groups, if possible from the same rats and at the same intervals as for the haematological examination. The determinations should be made on samples from either individual animals or on a pooled sample/sex/group.

c) Post-mortem examinations. A complete gross examination of all animals in the experiment should be made.

A limited number of organs should be weighed from at least 10 animals/sex/group (OECD; EEC; EPA, TSCA and FIFRA). The Japan/MAFF requires that organs should be weighed from all animals of the main groups and at least 10 animals/sex of the satellite groups. It is not clearly specified whether this includes all animals which died or were killed in a moribund condition during the study.

The histopathology requirements are almost, but not exactly, identical in the various guidelines. Histopathology should be performed:

i) on all target organs and gross lesions (including tumours, and lesions suspected of being tumours) in all animals;
ii) on lungs, livers and kidneys of all animals (EPA, TSCA and FIFRA; Japan/MAFF).
iii) on all preserved organs and tissues of all animals that died or were sacrificed during the study;
iv) on all preserved organs and tissues of animals in the control and high dose group(s);
v) if an excessive number of early deaths, or other problems, occur in the high dose group, complete histopathology should be performed on the group at the next dose level;
vi) if the experiment gives evidence of a substantial alteration of the animals' normal longevity, or the induction of effects that might lead to a toxic/neoplastic response, the group at the next lower dose level should be examined.

6.3. Inconsistencies

There are some inconsistencies within some of the individual guidelines :

6.3.1. The OECD-guidelines require 10 animals/sex for the satellite control group on the one hand, and blood sampling from 20 animals/sex of all groups on the other.

6.3.2. For an assessment of chronic toxicity in a combined study the regulatory guidelines require at least one treated and one control satellite group on the one hand, and haematological examinations on 20 (OECD) or 10 (EEC; Japan/MAFF; EPA, TSCA and FIFRA) rats/sex of all groups on the other. To take these differing requirements into account would mean that either animals scheduled for the carcinogenicity part of the study have to be

taken for blood examinations, which might affect some blood parameters (e.g. proliferative response of bone marrow or immune status) of these animals, or that the number of satellite groups has to be increased to such an extent that there are satellite groups for all dose levels.

6.3.3. According to the OECD, EEC and EPA/TSCA guidelines, the haematological examinations should be performed at 3 months, 6 months, at approximately 6-month intervals thereafter and at termination, while clinical chemistry determinations should be carried out at 6-month intervals and at termination (OECD, EEC), or just prior to the initiation of the study, near the middle and at the end of the dosing period (EPA/TSCA). In both cases the fact that haematological and clinical chemistry investigations are, in practice, carried out at the same time of sampling is not taken into consideration.

6.3.4. Differential blood counts are required on animals of the control and highest dose groups, but it is not specified whether it is the highest dose group from the satellite animals or the highest dose group from the animals scheduled for the carcinogenicity assessment. If clinical observations suggest a deterioration in the health of animals during the study, a differential blood count of the affected animals (from animals scheduled for chronic toxicity and carcinogenicity evaluation?) is required.

6.3.5. Both EPA guidelines require, for the selection of the intermediate dose level, that: "Ideally the intermediate dose level(s) should produce minimal observable toxic effects" and for the high dose level, that "...it should elicit signs of toxicity...". These requirements are correct for the chronic toxicity assessment but not necessarily for the carcinogenicity assessment because, for the latter, the high dose "should elicit signs of minimal toxicity" (see EPA/TSCA and FIFRA carcinogenicity guidelines).

6.3.6. The TGs emphasise the preference for the rat as the species for combined chronic toxicity-carcinogenicity studies and the texts of these guidelines consistently refer to the use of the rat or other rodents. However, there is an inconsistency in the OECD TG in the case of organ weight measurements where reference is also made to non-rodents. A

similar inconsistency exists in the EPA/TSCA and Japan/MAFF TGs for histopathology where reference is again made to non-rodents.

7. REPRODUCTIVE TOXICITY

7.1. Introduction

The purpose of reproductive toxicity studies in mammals is to establish whether exposure to a chemical affects fertility, and prenatal and/or postnatal development (ECETOC, 1983). For assessing such effects there are several methods which differ with regard to the periods of exposure. In the so-called teratogenicity studies, (cf. D. Recommendations - 7) animals are exposed during a defined period of pregnancy to assess the influence of the chemical on the intra-uterine development of the conceptus. All authorities have published guidelines for teratogenicity studies (preferably oral). EPA/TSCA (1983-1984) has now issued an additional guideline for an "Inhalation Developmental Toxicity Study". In one- or two-generation reproductive toxicity studies according to OECD and EEC, the parental animals and their progeny are exposed throughout their entire reproductive lifespan. EPA (TSCA and FIFRA) and Japan/MAFF have no guideline for a one-generation study, and the UK/HSC does not specify a two-generation study.

7.2. Teratogenicity studies

7.2.1. Animal criteria

a) Species. In all guidelines studies on at least two species, i.e. a rodent and a non-rodent, are required. The rat is the preferred rodent, but the mouse and hamster are mentioned as suitable alternatives. The rabbit (a lagomorph) is the unanimously recommended non-rodent.

Commonly-used laboratory animal strains should be employed in all guidelines. The strains "should not have low fecundity". The animals should be responsive to teratogens or "developmental toxins" (EPA/TSCA).

b) Age at start of study. Young mature virgin females of comparable age and size should be used. They should be acclimatized for at least five days prior to the start of study (OECD, EEC, UK/HSC). EPA (TSCA and

FIFRA) and Japan/MAFF require young pregnant animals and do not consider an acclimatisation period.

c) Group size. All guidelines require at least 20 pregnant rats, mice or hamsters, or 12 pregnant rabbits, per group. The objective is to ensure that the number of pups and (in the case of EEC and UK/HSC) litters, is sufficient to permit a valid evaluation.

d) Caging. The animals should be caged individually (OECD, EEC, UK/HSC; and EPA/TSCA for inhalation studies only).

7.2.2. Treatment

a) Dose levels/No. of control groups. Groups of at least three dose levels and a control group are required. EPA (TSCA and FIFRA) require a concurrent untreated or sham-treated control group. If the test substance is administered in a vehicle, OECD, EEC, and UK/HSC also recommend a vehicle control group. If a vehicle is used, it should be non-toxic or its toxicological properties should be understood. It should not be teratogenic or affect reproduction.

The dose levels are set as follows :

i) The low dose level should have no observable effects attributable to the test substance.

ii) The intermediate dose level is specified as the geometric mean between the low and high dose levels (OECD, EEC and UK/HSC). EPA/TSCA specifies that, ideally, this dose should cause minimal toxic effects in the mother. It also requires that if more than one intermediate dose level is used, the concentrations should be spaced to produce a gradation of toxic effects.

iii) The high dose level, unless limited by the physical/chemical nature or biological effects of the test substance, should ideally induce overt maternal toxicity, e.g. weight loss. There should be no more than 10% of maternal deaths .

If, in a "limit test", a dose level of at least 1000 mg/kgbw orally, or 5 mg/l by inhalation, produces no evidence of embryotoxicity or teratogenicity, studies at other dose levels may not be necessary.

b) Route of administration. All guidelines recommend administration by stomach tube (gavage). With the exception of Japan/MAFF, other routes of exposure are also acceptable as alternatives if they represent better the likely routes of human exposure. The test substance should be administered at approximately the same time each day. In inhalation studies whole-body exposure is preferred.

c) Duration of treatment. Treatment of pregnant females should cover the period of major organogenesis. The treatment period is the same in all guidelines. Japan/MAFF mentions an additional treatment period for the rat. Alternatively, the dosing period may be extended up to approximately one day before the expected date of delivery. The day on which vaginal plug and/or spermatozoa are observed is designated day 0 of pregnancy. If, alternatively, day 0 is based on the observation of mating or on artificial insemination, OECD, EEC, UK/HSC, EPA/FIFRA and Japan/MAFF require postponement of the beginning and the end of the treatment period by one day.

d) Frequency of dosing. The animals should be dosed daily during the treatment period, in inhalation studies for at least 6 hours per day.

7.2.3. Study observations

a) Clinical data

i) Body weight and food consumption should be measured weekly.

ii) Clinical signs and mortality. Observations for signs of toxicity, including time of onset, degree and duration, are required at least once per day, except in the EPA/FIFRA guideline which requires observation at least weekly. Additional daily observations should be made and appropriate actions taken to minimise loss of animals. Post-treatment observation should be continued until one day prior to term (OECD, EEC and UK/HSC). All guidelines except Japan/MAFF include a list of clinical parameters to be examined. Females showing signs of abortion or premature delivery, or which are moribund, should be sacrificed.

b) Post-mortem examinations

i) Gross pathology. Most of the guidelines require that all animals which are found dead during the study or are sacrificed during,

or at the end of, the test period should be examined macroscopically for structural or pathological changes which may have influenced pregnancy.

ii) Examination after Caesarean section or death. At necropsy the uterus should be removed. The number of live foetuses and embryonic or foetal deaths should be determined. The time of death in utero should be estimated. In addition, counting of corpora lutea in rats and rabbits is recommended. The weight of the gravid uterus should be recorded (EPA/TSCA).

iii) Examination of foetuses. All foetuses should be externally examined. Their sex, and individual body weights or the litter weights (EPA/FIFRA and Japan/MAFF), should be determined.

iv) Preparation and morphological examination of foetuses. In rodents, one-third to one-half of each litter should be prepared and examined for skeletal anomalies and the remainder prepared for examination of soft-tissue anomalies. In non-rodents, each foetus should be examined for visceral anomalies by dissection, and subsequently processed and examined for skeletal anomalies. According to UK/HSC the examination of the foetuses should include growth retardation, delayed ossification and haemorrhages.

7.3. One- and Two-generation Studies

7.3.1. Animal criteria

a) Species. The studies should preferably be conducted with rats. The mouse is also mentioned as a suitable species (OECD, EEC, EPA/FIFRA). If other species are used, the reason(s) for their selection should be provided (EPA, TSCA and FIFRA). Strains with low fecundity should not be used.

b) Age at start of study. Males should be between 5 and 9 weeks old (OECD and EEC), about 8 weeks old (EPA, TSCA and FIFRA) or weaned (Japan/MAFF). Females should be about 8 weeks old (EPA, TSCA and FIFRA), or weaned (EEC and Japan/MAFF). All animals should be acclimatized for approximately one week prior to the start of the study (OECD, EEC, Japan/MAFF).

c) Group size. Most TGs require sufficient animals to yield about 20 pregnant females at or near term, whereas UK/HSC recommends 20 litters. EPA (TSCA and FIFRA) and Japan/MAFF require at least 20 males. The OECD, EEC and UK/HSC guidelines give no precise information on the number of males. This can be calculated according to a mating ratio of 1 male to 1 or 2 females.

d) Caging. All guidelines, except UK/HSC (which does not mention it), require separate caging only for pregnant females or females near parturition.

7.3.2. Treatment

a) Dose levels/No. of control groups. At least three dose levels and a control group are required. EPA (TSCA and FIFRA) require a concurrent control group. A control group receiving the vehicle in the largest volume, as at the highest dose level, should be included if the test substance is administered in a vehicle. The vehicle should be without toxic effects. If a test substance causes reduced dietary intake, the use of a paired-fed control may be considered necessary (OECD, EEC). The following criteria are also specified :

i) Ideally the low dose should not be expected to show any evidence of toxicity to parent animals and offspring.

ii) For the intermediate dose(s) there should, ideally, be only minimal toxic effects attributable to the test substance (OECD; EEC; EPA, TSCA and FIFRA; Japan/MAFF).

iii) Unless limited by the physical/chemical nature or biological effects of the substance, the high dose level should ideally induce toxic effects but no mortality in the parent (P) animals.

According to the EEC guidelines for one- and two-generation studies, and the OECD guidelines for one-generation studies, if the test compound produces no evidence of toxic effects at a dose level of at least 1000 mg/kgbw ("limit test") then studies at other dose levels should not be considered necessary.

b) Route of administration. The chemical should be administered in the diet or drinking water. Other suitable routes are also acceptable. If

the dermal or inhalation route of administration is used, justification for its selection should be provided (EPA/TSCA).

c) Duration of treatment. Male rats of the P-generation should be treated for at least one complete spermatogenic cycle, i.e. 8 to 10 weeks prior to the mating period, while male mice should be treated for 8 weeks. Treatment should be continued throughout the mating period (UK/HSC; EPA, TSCA and FIFRA) and up to the weaning of the F_1 offspring (Japan/MAFF). The females of the P-generation must be treated for at least two complete oestrous cycles, i.e. 2 or 3 weeks prior to mating and throughout the mating, pregnancy and lactation periods until the F_1 offspring are weaned. EPA (TSCA and FIFRA) and Japan/MAFF require treatment of the females for at least 8 weeks. Treatment of males and females of the F_1 generation should start after weaning and end with their sacrifice.

d) Frequency of dosing. Continuous dosing in the diet or drinking water, or daily dosing by gavage or with capsules, is required. In the latter case the test substance should be administered at approximately the same time each day and the dose should be adjusted periodically according to body weight.

7.3.3. Procedure

a) Mating

i) P generation. All guidelines except those of the UK/HSC (not mentioned) require mating of one female and one male until the female is pregnant or 3 weeks have elapsed (failure to conceive). The OECD and EEC guidelines also permit mating of 1 male with 2 females. During the mating period the female should always be mated with the same partner.

ii) F_1 generation. In all two-generation studies, one male and one female of each litter are selected at random for cross mating with the young of another litter of the same dose group. The mating period starts when rats are at least 13 (OECD, EEC) or approximately 17 (EPA/FIFRA) weeks old and mice are at least 11 weeks old.

iii) The possibility of using a second litter is mentioned in the OECD

guideline for the two-generation study and in the UK/HSC guideline for the one-generation study.

b) Proof of fertility. The females should be examined each morning for the presence of sperm and/or vaginal plugs. The day on which the females are impregnated is designated day 0 of pregnancy. Partners which fail to mate should be mated with sires or dams of proven fertility. All guidelines except UK/HSC state that pairs which fail to mate should be examined to determine the cause of the apparent infertility. This may involve such procedures as additional opportunities to mate with other proven sires or dams, examination of the oestrous cycle or spermatogenesis, and microscopic examination of the reproductive organs.

c) Rearing. The dams are allowed to litter normally and rear their progeny to the stage of weaning (OECD, EEC, UK/HSC). The OECD and EEC also recommend rearing litters of standardised size. In this case the litter size is reduced to 4 male and 4 female young between days 1 and 4 (EEC), or on day 4 (OECD), after birth. EPA (TSCA and FIFRA) and Japan/MAFF mention only "standardised rearing" in the same manner as OECD. In some guidelines (OECD for both one- and two-generation studies, EEC and EPA/TSCA for two-generation studies) the adjustment of litters of less than 8, or the elimination of runts only, is not considered appropriate.

7.3.4. Study observations

a) Clinical data

i) Body weight. The parental animals should be weighed on the first day of dosing and weekly thereafter.

ii) Food consumption. Measurement of food consumption is specified only by OECD and EEC. The OECD guidelines for both studies and the EEC guidelines for two-generation studies recommend weekly measurement, with optional daily measurements during pregnancy. For one-generation studies, the EEC recommends daily measurements until mating and weekly measurements after parturition, but measurements during pregnancy are not mentioned.

iii) Clinical examination. The general condition of the animals

should be checked at least once daily throughout the study period. Changes in behaviour, signs of difficult or prolonged parturition, signs of toxicity, and mortality should be recorded.

The UK/HSC guidelines do not mention the above three items.

b) Examination of litters. As soon as possible after delivery, the number of stillborn and live young, the sex (only OECD and EEC) of the young and the presence of gross anomalies should be examined. Most guidelines require that dead and moribund pups, and pups sacrificed on day 4 after birth for litter standardisation, be examined for possible defects. Live offspring should be counted and weighed directly after birth, or on the morning after birth; on other days defined in the TGs; and thereafter weekly up to the end of the study. Most guidelines require a record of physical or behavioural changes in dams and offspring. The UK/HSC mentions observation of postnatal development, without further details.

c) Dates of sacrifice. According to most guidelines, parental males of the P and F_1 generations should be sacrificed at the end of the mating period or if they are no longer necessary for the assessment of reproductive effects. The parent (P) and F_1 females selected for mating should be sacrificed after weaning of their offspring (EPA, TSCA and FIFRA; Japan/MAFF) or when they are no longer needed for the assessment of their reproductive performance (OECD, EEC). F_1 animals not selected for mating and F_2 offspring should be sacrificed after weaning. UK/HSC does not specify dates of sacrifice.

d) Post-mortem examinations.

i) Gross pathology. After sacrifice or intercurrent death, all animals should be examined macroscopically and particular attention paid to the reproductive organs. The OECD and EEC guidelines for one-generation studies require preservation of the target and reproductive organs of all parental animals. For two-generation studies the TGs require preservation of these organs from animals selected for mating, while Japan/MAFF

requires preservation of the reproductive organs of all animals.

ii) Histopathology. In most guidelines, the selection of organs to be examined in all parental P and F_1 animals is almost identical. Generally, the animals of the highest dose and control groups have to be examined histopathologically, although OECD and EEC state that this should be undertaken only if the organs have not been examined in other multiple-dose studies. In the two-generation studies, histopathology is limited to the animals selected for mating. When gross pathological changes are detected, the organs and tissues of all animals in all dose groups should be examined microscopically. The OECD and EEC also recommend examination of all animals which die intercurrently, and microscopic examination of the reproductive organs of animals suspected of being infertile.

C. DIFFERENCES BETWEEN OECD AND OTHER GUIDELINES

1. SUBCHRONIC TOXICITY STUDIES (see Appendix 2)

1.1. Animal criteria - species. EPA/TSCA and EPA/FIFRA require rodent species, preferably the rat, to be used in inhalation studies, and require justification for the use of another mammalian species, which implies that non-rodents are also allowed.

1.2. Clinical data

a) Food consumption. In the text of some guidelines (including OECD) it is stated that there is no need to measure food consumption if the test substance is administered in the drinking water. Other guidelines require the weekly measurement of food consumption in all cases.

b) Ophthalmology. The number of animals per group, number of groups and/or time of investigation in the EPA/TSCA and Japan/MAFF requirements differ from those of the OECD TGs.

1.3. Haematology, clinical chemistry and urinalysis. The number of animals per group and the time of investigation for haematology and clinical chemistry in the EPA/TSCA requirements differ from those of the OECD TGs.

1.4. Post-mortem examinations. EPA (TSCA and FIFRA) and Japan/MAFF require full histopathology on all rodents that died or were killed during the study. In the various subchronic studies there are many discrepancies concerning the organs to be routinely investigated microscopically, or after indications of signs of toxicity or target organ involvement.

The OECD, EEC and UK/HSC restrict the histopathological examination of non-rodents initially to animals of the control and high dose groups, whereas the other TGs require this for all non-rodents of all groups.

2. CHRONIC TOXICITY STUDIES

There are several differences in detail as can be seen from Appendix 3. The major ones are as follows.

2.1. Species. The OECD does not see the need for a second species unless circumstances require it. The EPA (TSCA and FIFRA) and Japan/MAFF specifically request a second species (dog preferred) for chronic toxicity testing.

2.2. Duration of treatment. Japan/MAFF advocates a period of longer than 12 months for rodents, e.g. 18 months for mice or 24 months for rats.

2.3. Food and water consumption. While OECD and Japan/MAFF mention measurements of only food intake, EEC asks for measurements of food intake and water consumption when the test substance is administered in the drinking water. EPA/TSCA asks for food or water consumption depending on the route of administration, while EPA/FIFRA does not specify this exactly.

2.4. Haematology,clinical chemistry and urinalysis. There is some inconsistency within the OECD guidelines in that the frequency of testing for blood clinical chemistry (every 6 months) is different from that for haematology and urinalysis (3 and 6 months, and then every 6 months). Normally, EPA/FIFRA and Japan/MAFF do not require blood sampling for haematology and urinalysis at 3 months. EPA (TSCA and FIFRA) do not specify exact timings

for clinical chemistry analysis but use the terms "prior to initiation of dosing", "near the middle" and "at the end of the test period"(TSCA) and "at the beginning, at the middle and at the end of the test period"(FIFRA).

2.5. Organ weights. There are some differences between guidelines concerning the tissues to be weighed. For rodents and non-rodents, EPA/FIFRA does not require weighing of the adrenals. Also, EPA/FIFRA and Japan/MAFF require only the testes to be weighed, in contrast to the other TGs which require the weights of gonads. For non-rodents EPA/FIFRA does not require thyroids to be weighed. It is not clearly specified whether these determinations should also be made on all animals which died or were killed in a moribund condition during the study.

2.6. Histopathology - EPA (TSCA and FIFRA) and Japan/MAFF require organs and tissues from all non-rodents to be examined microscopically, in contrast to the more limited requirement of OECD and EEC, i.e. examination of control and high dose animals plus any which died or were sacrificed during the study. Japan/MAFF and EPA (TSCA and FIFRA) require examination of lungs, liver and kidneys of all rodents.

3. CARCINOGENICITY STUDIES (see Appendix 4)
A review of the five TGs shows an overall comparability. The differences that do occur can be explained by the different regulatory status of the guidelines (cf. section B.1).

3.1. Species/strain. The preferred strain in the OECD TG is one with a low incidence of spontaneous tumours. In the UK/HSC guideline, inbred or F_1 hybrid strains are also accepted. The more practical phrase "commonly used laboratory strain" covers the actual situation in each laboratory.

3.2. Size of groups. EPA/FIFRA and Japan/MAFF indicate under "number of animals" that 50% or more should survive after 15 months in mice and 18 months in rats, and 25% or more at 18 months for mice and 24 months for rats. It is not made clear whether this provision relates to interim sacrifices, dose selection, or is a validation criterion for the test.

3.3. Route of administration. Both EPA TGs describe dermal administration in some detail, while the others do not give details of this route or do not mention it.

3.4. Food and water consumption. While OECD and Japan/MAFF mention measurements of only food intake, EEC asks for measurements of food intake and water consumption when the test substance is administered in the drinking water. EPA/TSCA asks for food or water consumption depending on the route of administration, while EPA/FIFRA does not specify this exactly.

3.5. Haematology. EPA/FIFRA require a blood smear from 10 animals/sex/group. The other TGs require it for all animals.

3.6. Organ weights. Only EPA/FIFRA and Japan/MAFF request organ weights for liver, kidneys, brain and testes for 10 animals/sex/group.

3.7. Histopathology. Only Japan/MAFF and EPA/FIFRA ask for an inspection of lungs, liver and kidneys of all animals.

4. COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDIES (see Appendix 5).

4.1. Number of animals in the satellite groups. While OECD and EEC ask for 10 males and 10 females in the satellite control group and 20 males and 20 females in the satellite dose group(s), EPA/TSCA and Japan/MAFF require 20 males and 20 females in all satellite groups. EPA/FIFRA requires 10 males and 10 females in the satellite control group, but does not exactly specify the number in the satellite dose group(s).

4.2. Duration of treatment for the satellite groups. Japan/MAFF suggests a dosing period of 24 months (rat) or 18 months (mouse) for the satellite animals. EPA/FIFRA asks for a duration of at least 24 months in the special case of food additives.

4.3. Food and water consumption. While OECD and Japan/MAFF mention measurements of only food intake, EEC asks for measurements of food intake and water consumption when the test substance is administered in the drinking water. EPA/TSCA asks for food or water consumption depending on the route of administration, while EPA/FIFRA does not specify this exactly.

4.4. Haematological examinations. These are performed on blood samples in 10 rats/sex of all groups (EEC, EPA/FIFRA, Japan/MAFF).

4.5. Blood sampling and urine collection. EPA/FIFRA and Japan/MAFF require blood sampling and urine collection for the first time after approximately 6-months study duration. If haematological effects are found in the subchronic toxicity study, JAPAN/MAFF requires an additional examination after 3 months.

4.6. Clinical chemistry determinations. EPA/TSCA requires only 3 clinical chemical determinations, one just prior to the initiation of dosing (base-line data), and one near the middle and another at the end of the test period.

4.7. Organ weights. There are some differences between the guidelines in the detailed tissues to be weighed at necropsy. EPA/FIFRA does not require adrenals, and EPA/FIFRA and Japan/MAFF require (from the gonads) only the testes, in contrast to all other guidelines which require testes and ovaries. Japan/MAFF requires organ weights from all animals of the main groups and from at least 10 animals/sex of the satellite groups.

5. REPRODUCTIVE TOXICITY STUDIES

There are some differences between the various guidelines as already listed in Appendices 6-7-8 and discussed above. Some TGs give detailed instructions about individual parameters, whereas other guidelines fail to mention some of them or do not specify them exactly. Only the major differences between the OECD and other guidelines are outlined below.

5.1. Teratogenicity (Appendix 7)

In contrast to the other authorities, the EPA/TSCA guideline in its latest 1984 version designates this study as a "Developmental Toxicity Study". For this reason the adverse effect on the conceptus is defined as developmental toxicity in Appendix 7.

5.1.1. Animal criteria; size of groups. The EEC and UK/HSC guidelines require the use of a sufficient number of pregnant animals to ensure that the number of pups and litters produced is sufficient for evaluating the teratogenic potential of the substance.

5.1.2. Treatment; dose levels / No. of groups. EPA (TSCA and FIFRA) and Japan/MAFF require either a vehicle control group if the test substance is administered in a vehicle or an untreated or sham-treated control. EPA (TSCA and FIFRA) require a concurrent control or vehicle control group. According to the EPA/TSCA guideline, the females treated with the intermediate dose(s) should ideally show minimal observable toxic effects.

5.1.3. Study observations; clinical signs/mortality. According to EPA/FIFRA not more than 10% of animals in any test group should be lost by cannibalism.

5.1.4. Post-mortem examinations; examinations after Caesarean section or death. EPA/TSCA additionally requires weighing of the uterus.

5.2. One- and two-generation studies (Appendices 8-9)

The UK/HSC has not published a guideline for two-generation studies but mentions that in some circumstances two- or three-generation studies may be carried out. EPA (TSCA and FIFRA) and Japan/MAFF have no guideline for one-generation studies. EEC and Japan/MAFF mention the possibility of three-generation studies. There is a difference in terminology in the headings of these TGs. In contrast to OECD, EPA (TSCA and FIFRA) designate the two-generation study as "Reproduction and Fertility Effects" and Japan/MAFF as "Reproduction Study".

5.2.1. Animal criteria.

a) Age at start of study. All TGs, except the OECD guideline for two-generation studies, specify the initial age of the female animals.

b) Size of group. The EPA (TSCA and FIFRA) and Japan/MAFF guidelines specify the number of males per group. The UK/HSC defines the size of groups by the number of litters and not by the number of pregnant females.

5.2.2. Treatment

a) Dose levels/No. of groups.

i) EPA (TSCA and FIFRA) guidelines for two-generation studies state that where there is a "usable" estimation of human exposure, the lowest dose should exceed this.

ii) For two-generation studies the EPA (TSCA and FIFRA) guidelines additionally recommend that if more than one intermediate dose is administered they should be spaced to produce a gradation of toxic effects.

iii) According to the EPA TGs the highest concentration of the test compound in the diet should not exceed 5%. In the Japan/MAFF guideline only the toxicity to the dams is considered for the choice of the highest concentration.

b) Treatment period. EPA (TSCA and FIFRA) and Japan/MAFF mention a shorter duration of treatment of the males of the P-generation prior to mating but also treatment throughout the mating period. Treatment of males during the mating period is also recommended by the UK/HSC guidelines (in which "breeding cycle" is added in brackets but no definition of this is given). In females the treatment prior to mating should be prolonged to 8 weeks according to EPA (TSCA and FIFRA) and Japan/MAFF.

Japan/MAFF recommends, when deemed necessary, treating the animals of the F_2-generation after weaning.

c) Rearing. EPA (TSCA and FIFRA) and Japan/MAFF recommend the rearing of litters of standardised size. If litter sizes are standardised, the EEC in both guidelines recommends selection of the pups for further rearing between day 1 and 4, but the other guidelines on day 4 after birth.

5.2.3. Study observations; examination during lactation. In the EPA/TSCA and Japan/MAFF guidelines, individual weighing of the pups is required.

D. RECOMMENDATIONS FOR HARMONISATION

1. GENERAL RECOMMENDATIONS

The objectives of a toxicity study will vary according to the intended use of, the expected human exposure to, and the physico-chemical properties of, the test substance. There is thus a need for sufficient flexibility in the TGs to allow these to be taken into account when planning, executing and

reporting a study. The TGs should be sufficiently detailed to give guidance on the overall design of a study and the individual TGs should be consistent as far as possible on these points, i.e. they should be harmonised. Variations should be allowed provided that the reasons for them are explained.

The OECD TGs and the Principles of Good Laboratory Practice (OECD, 1981-a) provided the basis for an agreement on the Mutual Acceptance of Data and on May 12th, 1981 were adopted by the OECD Council representing 24 member countries (OECD, 1981-b). Although the OECD TGs are considered to be the most appropriate basis for comparisons it is apparent that they need updating. During this updating the OECD should consider the improvements introduced in other TGs (e.g. EEC). When a TG for individual studies is redrafted, more consideration should be given to its relationship with the other study designs.

The Task Force recognises that there are some areas in the TGs requiring scientific comment on their relevance (e.g. some of the biochemical parameters), but it was felt that this did not come within its remit for making recommendations.

2. RECOMMENDATIONS FOR REQUIREMENTS COMMON TO ALL TYPES OF TOXICITY STUDIES

A revision of guidelines is recommended to provide a more concise structure, clearer layout and more precise wording. Contradictions within the same TGs should be avoided.

The general requirements common to all toxicity TGs should be specifically outlined in a separate chapter (see section B.2). In those cases where there is a specific deviation from the normal procedure (e.g. caging for inhalation, dermal or reproductive toxicity studies, etc.) this can be detailed in the individual TGs.

2.1. Animal Criteria

a) Age at start of exposure. For oral studies with rodents, all TGs should adopt the following version whatever the duration of the study: "Rats should be taken as soon as possible after weaning, preferably at before 6 weeks, but not later than 8 weeks, of age. The weight difference between groups at the start should not exceed ±20%, and the

animals should be acclimatised for at least 5 days prior to the study".

b) Caging. The OECD gives quite detailed recommendations about housing conditions in their individual guidelines and it is recommended that these are followed in the other TGs.

c) Environmental conditions. The recommendations of the OECD TGs for rodents in subchronic toxicity studies (i.e. relative humidity 30-70%, temp. 20±2°C, 12h light, 12h dark) should be adopted by, and/or amended in, the other TGs. For inhalation studies some additional requirements may be necessary as already detailed in the OECD TGs for long-term studies (cf. Appendix 6).

2.2. Treatment

a) Requirements for test compound. Ideally "toxicity testing should be done with the technical grade product or with the technical grade of active ingredients in the product"(OECD). However, some toxicity studies are carried out at an early stage in the development of a chemical and the tested substance may not be the final technical grade product at this stage. Such studies may however give results which are relevant to the toxicity of the technical grade products, and it is recommended that the TGs take this situation into account.

b) Route of administration. It is recommended that the OECD requirements for the subchronic toxicity TGs are adopted in all TGs.

c) Frequency of dosing. It is recommended that the OECD TGs should be followed, with allowance for specific exposure conditions where necessary.

2.3. Study Observations

a) Clinical data

i) Body weight. It is recommended that for all types of study, body weight should be measured at least weekly for the first 13 weeks and then at least every 4 weeks.

ii) Food and water consumption. It is recommended that food

consumption should be measured at least weekly for the first 13 weeks, then at least every 4 weeks when the test substance is administered in food. If the test substance is administered in drinking water, the water consumption should be measured at the same intervals.

b) Haematology, clinical chemistry and urinalysis. The frequency of the investigations, the number of groups and the number of animals per group should be harmonised. A minimum core of assays is recommended. Although it is not appropriate to nominate the numbers and types of assays in the core it should be noted that in those studies where it is relevant the TGs advocate a minimum of about 6 assays each for haematology and clinical chemistry and about 8 assays for urinalysis. Additional assays, if needed, should be left to the discretion of the study director.

c) Post-mortem examinations.

i) The specification of a basic set of organs for weighing and histopathology is recommended, such examination of any others being optional according to the needs of the experiment.

ii) It is recommended that the histopathology in non-rodent studies should be restricted initially to the high dose and control animals.

3. RECOMMENDATIONS FOR SUBCHRONIC TOXICITY STUDIES (cf. also D-2)

3.1. Major Recommendation; Study Duration.

Most guidelines refer to 90-day studies for oral administration or inhalation exposure. OECD, EEC and UK/HSC also have separate guidelines for treatments of shorter duration. It is recommended that 28-day subchronic studies giving clear-cut results with rodents are accepted by regulatory bodies as an alternative to 90-day studies. This will help to reduce the number of animals and the costs involved. In some cases the shorter studies will provide sufficient information commensurate with the nature and the future use of a chemical (Cohen, 1982). In fact, the UK/HSC (1977) normally requires 28-day studies and lists 90-day studies only as an additional requirement (e.g. for chemicals with a long biological half-life, or where it is suspected that toxic effects may become apparent after more prolonged

exposure). 28-day studies are likely to provide adequate information on the effects of repeated exposure for most chemicals.

3.2. Other Recommendations.

There are several aspects in most TGs (including those of the OECD) which should be made clearer.

a) When a recovery period is included for a group at the high dose level, a concurrent control group is needed.

b) Limit tests for substances of low toxicity, as in the other guidelines, should be incorporated into the Japan/MAFF guidelines.

c) The choice of animal species for inhalation studies should remain flexible, as in the OECD TG.

d) It should be made clear that organ weights should be measured at scheduled kills only.

e) The list of organs to be investigated by histopathology should be harmonised within and between the different TGs. A clear distinction should be made between organs to be investigated routinely and those which should be studied only if there is an indication of toxicity or target organ involvement.

4. RECOMMENDATIONS FOR CHRONIC TOXICITY STUDIES (cf. also D-2)

a) Requirement for Chronic Evaluation.

This evaluation should be treated as a separate entity from carcinogenicity studies. In rodent studies the evaluation should be undertaken either in separately-identified satellite groups alongside the carcinogenicity evaluation or should form a separate study (cf. 6-1 Combined Studies).

b) Species tested.

The OECD requirement for using the rat should be generally adopted. The need for a second species should be examined on a case by case basis and should not be mandatory.

c) Study duration.

There is no need for the dog study to last for 12 months since there is enough evidence to show that a duration of "at least six months" is sufficient in this species (GIFAP, 1982).

In studies with rodents, Japan/MAFF requires 18 months for the mouse or two years for the rat. This should be brought into line with the minimum requirement of 12 months accepted by the other guidelines.

d) Haematology/clinical chemical/urinalysis.
It is recommended that sampling take place at the same time interval for these assays and that they should be carried out usually six months after the start of the study and thereafter at six-monthly intervals and at termination.

e) Organ weights.
It should be made clear that organ weights only need to be determined at scheduled kills.

5. RECOMMENDATIONS FOR CARCINOGENICITY STUDIES (cf. also D-2)
At present, the TGs in use (and reviewed here) are in accordance with the OECD TG and contain only differences which will most probably not lead to the rejection of the study in another country. However, the evaluation and interpretation of the results can give rise to extended discussions with government, and other, experts. Discussions on the preferred strain, diet, housing, etc. are still going on. It is not expected that complete agreement will be reached in the near future. The following recommendations can, however, be made.

a) The studies should be carried out with strains having a low and stable tumour incidence profile, in laboratories which have sufficient background data on that particular strain.

b) Some of the guidelines specify the use of two species. The decision to carry out studies on two species should be based on hazard assessment considerations and should not be a fixed requirement. If such a study is needed, its choice should be justified by a consideration of all available information.

c) The requirement concerning the number of animals per dose group and the percentage of survivors in EPA/FIFRA and Japan/MAFF should be put into the section on duration of study, as in the OECD TGs.

d) It is recommended that the requirements for histopathology examination follow those in the OECD TGs. Organ weight measurements should not be required.

6. RECOMMENDATIONS FOR COMBINED CHRONIC TOXICITY-CARCINOGENICITY STUDIES (cf. also D.2)
Although the various regulatory organisations have given the option of performing combined studies in order to make the most efficient use of laboratory animals, facilities and time, and to reduce costs, these

objectives are not always compatible with the toxicological objectives of either carcinogenicity or chronic toxicity studies. This conflict of objectives is borne out by the somewhat confusing details on some aspects of these studies, which differ within and between TGs. This is particularly noticeable in the numbers of animals recommended for control and treated groups of the satellite animals (C.4.1), the numbers of animals recommended for blood sampling in the satellite groups (B.6.3.1 and 6.3.2) and some requirements for the dose levels (B.6.3.5). Moreover, if it is necessary to include animals in satellite groups at all dose levels, the differences in efficiency and costs compared with the performance of two separate studies become very small.

6.1. General Recommendations for Revision of Existing Guidelines

In view of the discrepancies and the questionable effectiveness in reducing costs or the use of resources, as discussed above, two alternatives could be recommended.

a) Revised guidelines. A revised OECD guideline should clearly designate which animals are scheduled for carcinogenicity assessment and which for the chronic toxicity part of the study. It should be made clear which observations should be carried out, and on which animals (main groups or satellite groups).

b) Conduct of two separate experiments. The alternative is to carry out two separate experiments, one for determining chronic toxicity and the other for assessing carcinogenicity. The costs would not be much higher because feed mixing, study observations, analytical investigations, etc. could be done in parallel. The number of animals used would scarcely exceed the number in a properly-designed combined chronic toxicity/carcinogenicity study.

6.2. Specific Recommendations (see D-4 Chronic Toxicity and D-5 Carcinogenicity, above).

7. RECOMMENDATIONS FOR REPRODUCTIVE TOXICITY STUDIES

7.1. Major Recommendations

In all reproductive toxicity guidelines the same terminology should be used in describing these studies. Studies in which the dams are exposed to a chemical during organogenesis of the conceptus are at present referred to

as "teratogenicity studies" in most guidelines. These are more correctly designated "embryotoxicity studies" because teratogenic effects are only one part of the total spectrum of the embryotoxic effects to be studied (ECETOC, 1983). Studies where the animals are exposed during the whole of the reproductive cycle over one or two generations should be called "fertility and general reproductive performance studies" with an indication of the number of generations involved.

7.2. Other Recommendations

7.2.1. Embryotoxicity studies

Two control groups (i.e. an untreated or sham-treated control and a vehicle control) should not be required if the vehicle is known to be non-toxic at the dose given.

7.2.2. Fertility and general reproductive performance studies

a) Each dose group should consist of a definite number of females, as in the OECD TGs, because the number of pregnancies or litters may be influenced by the toxicity of the test compound.
b) The low dose should exceed the human exposure level, if this is known.
c) The treatment of the males prior to mating should be continued throughout the mating period in all TGs.
d) In all guidelines the duration of treatment of the females prior to mating should be harmonised to accord with that of both EPA (TSCA and FIFRA) and Japan/MAFF TGs.
e) During lactation the individual body weights of the pups should be determined according to the EPA/TSCA and Japan/MAFF TGs.
f) The rearing procedure for the progeny should be left to the discretion of the study director and not limited by standardising the litter size.

E. APPENDICES

REGULATORY GUIDELINES FOR CONDUCTING TOXICITY STUDIES : COMPARISON WITH OECD TEST GUIDELINES

Appendix 1. Abbreviations in Tables

d	day
d. of gest.	day of gestation
F	females
h	hours
kgbw	kilogram body weight
m	month
mg	milligrams
M	males
P	parent
rel.hum.	relative humidity
wk	week(s)
=	text the same as or equivalent to OECD

A clear distinction has been made between the phrases "not mentioned" and "not specified" (the latter meaning: mentioned but without specifying details).

EEC : European Economic Communities.

EPA/TSCA : Environmental Protection Agency/Toxic Substances Control Act.

EPA/FIFRA : Environmental Protection Agency/Federal Insecticide, Fungicide and Rodenticide Act.

OECD : Organisation for Economic Co-operation and Development.

JAPAN/MAFF : Japan/Ministry of Agriculture, Forestry and Fisheries.

UK/HSC : United Kingdom/Health and Safety Commission.

APPENDIX 2

SUBCHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD NOS. 407, 408, 409

A. ORAL TREATMENT

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 407 TG 408 (rodent) TG 409 (non-rodent)	EEC (1983/84)	UK/HSC (1982) Test 3a (rodent) Additional tests 2a (rodent) 2d (non-rodent)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982) § 82-1	Japan/MAFF (1985)
ANIMALS						
1. Species/strain						
- rodents	preferably rat of commonly used strain	=	=	=	=	at least two mammalian species
- non-rodents	dog, preferably of defined breed	=	=	=	=	
2. Age at start of dosing						
- rodents	as soon as possible after weaning, ideally before 6 wk but not older than 8 wk	ideally before 6 wk, but not older than 8 wk	ideally before 6 wk, but not older than 8 wk	=	=	=
- non-rodents	preferably 4 - 6 m, but not older than 9 m	=	=	=	=	=
3. Size of groups						
- rodents	at least 5M/5F in 14/28-d study and 10M/10F in 90-d study; higher numbers if interim sacrifices are planned	at least 5M/5F in 28-d study and 10M/10F in 90-d study; higher numbers if interim sacrifices are planned	at least 5M/5F in 28-d study and 10M/10F in 90-d study; higher numbers if interim sacrifices are planned	at least 10M/10F; higher numbers if interim sacrifices are planned	at least 10M/10F; higher numbers if interim sacrifices are planned	at least 10 M/10 F; higher numbers if interim sacrifices are planned
- non-rodents	at least 4M/4F	=	=	= higher numbers if interim sacrifices are planned	= higher numbers if interim sacrifices are planned	= higher numbers if interim sacrifices are planned
4. Satellite group(s)						
- rodents	5M/5F in 14/28-d study, 10M/10F in 90-d study at the high dose level	5M/5F in 28-d study, 10M/10F in 90-d study at the high dose level	5M/5F in 28-d study, 10M/10F in 90-d study at the high dose level	10M/10F at the high dose level	10M/10F at the high dose level	10M/10F
- non-rodents	not mentioned	=	=	=	=	4M/4F
TREATMENT						
5. Dose levels	at least 3 + control	=	=	=	=	=
- low dose	no evidence of toxicity (but higher than estimated human exposure)	=	=	=	=	not specified
- intermediate dose(s)	minimal toxic effects	=	=	=	=	not specified
- high dose	toxic effects but not to many fatalities in rodents and no fatalities in non-rodents	=	=	=	=	=
- limit test	1000 mg/kg bw/d	=	=	=	=	not mentioned

APPENDIX 2

(continued 2)

SUBCHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD NOS. 407, 408, 409

A. ORAL TREATMENT

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983/84)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
6. Route of administration	oral (in diet, drinking water, by gavage or in capsules)	=	=	=	=	in diet (in principle)
7. Frequency of dosing	ideally 7 d/wk, 5 d/wk acceptable	ideally 7 d per wk, 5 d/wk* acceptable	ideally 7 d per wk	=	=	not mentioned
8. a) Duration of treatment - rodents	at least 14/28 d (407) or 90 d (408)	at least 28 d (1984) or 90 d (1983)	at least 28 d (3a) or 90 d (2a, 2d)	at least 90 d	at least 90 d	90 d
- non-rodents	at least 90 d	90 d	=	=	=	90 d
b) - duration of recovery period (if necessary)	14 (407) or 28 d (408)	14 d (1984) or 28 d (1983, rodents)	= (3a) or (2a)	not less than 28 d (rodents)	28 d (rodents)	28 d
STUDY OBSERVATIONS **I CLINICAL DATA**						
9. Body weights	weekly	=	=	=	=	=
10. Food consumption	weekly (except when test substance is administered in drinking water)	weekly	weekly	weekly when test substance is administered in food	=	weekly, including calculation of food efficiency during the growing period
11. Water consumption	weekly (when test substance is administered in drinking water)	weekly (only when test substance is administered in drinking water)	weekly (only when test substance is administered in drinking water)	=	=	not mentioned
12. Clinical signs	observe at least once daily	daily	daily	=	=	=
13. Mortality	observe at least twice daily	observe regularly	observe regularly	=	=	observe at least once daily

* 5 days per week scheme, not mentioned in 28-day study.

APPENDIX 2

(continued 3)

SUBCHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD NOS. 407, 408, 409

A. ORAL TREATMENT

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983/84)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
14. Ophthalmology - rodents	prior to start and at end of dosing at least in high dose and control animals*). If changes are detected, then all animals	=*)	=*)	prior to start and at end of dosing at least 5M/5F/sex/group	=	=
- non-rodents	prior to start and at end of dosing at least in high dose and control animals. If changes are detected, then all animals	=	=	prior to start and at end of dosing on all animals	=	prior to start and at end of dosing on all animals
II HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALYSIS (cf. cont. 8)						
15. Haematology - rodents	at end of the test period in all animals**)	=**)	=	at least 5M/5F/group shortly before start of dosing, after 30 d of dosing and at the end of the test period	=	=
- non-rodents	all animals at the beginning, then at monthly intervals or midway through the test period and at the end of the test period	=	=	all animals shortly before start of dosing, after 30 d of dosing and at the end of the test period	=	all animals before start, 1 - 2 interim examinations and at end of the test period
16. Clinical Chemistry - rodents	at end of the test period in all animals**)	=**)	=	at least 5M/5F/group shortly before start of dosing, after 30 d of dosing and at the end of the test period	=	at end of the test period on all animals
- non-rodents	all animals at the beginning, at monthly intervals or midway through the test period and at the end of test period after (max. 24 h) fasting	=	=	all animals shortly before start, after 30 d of dosing and at the end of test period after fasting not more than 24 h	=	all animals before start, 1 - 2 interim examinations and at end of the test period
17. Urinalysis	not required on routine basis	=	=	=	=	all animals at the end of test period if considered necessary

*) not required for 14/28-d studies (OECD; EEC; UK/HSC)

**) if historical baseline data are inadequate, then also prior to test for OECD (14/28/90-d rodent studies) and EEC (90-d rodent studies)

APPENDIX 2

(continued 4)

SUBCHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD NOS. 407, 408, 409

A. ORAL TREATMENT

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983/84)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
III POST MORTEM EXAMINATIONS (cf. cont. 9)						
18. Gross pathology	all animals	=	=	=	=	=
19. Organ weights	all animals	=	=	=	=	=
20. Histopathology - rodents	all animals of the high dose and control groups; gross lesions; target organs in all groups; kidneys, liver and lungs in low and intermediate dose groups*)	=**)	=**)	all animals of the high dose and control groups, and all animals that died or were killed during the study; gross lesions, target organs, and kidneys, liver and lungs in all dose groups	all animals of the high dose and control groups, and all animals that died or were killed during the study; gross lesions, target organs, and kidneys, liver and lungs in all dose groups	all animals of the high dose and control groups, and all animals that died or were killed during the study; gross lesions, target organs and kidneys, liver and lungs in all dose groups
- non-rodents	animals in the control and high dose groups; further histopathology in other groups necessary on organs which show lesions in the high dose group or for which clinical observations indicate such a need	=	=	all animals	all animals	all animals

*) Kidneys, liver and lungs are not obligatory in first instance in low and intermediate dose group(s) in 28-d study

**) Kidneys, liver, lungs and target organs are not obligatory in first instance in low and intermediate dose group(s) in 28-d study

APPENDIX 2

(continued 5)

SUBCHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD NOS. 410, 411

B. DERMAL TREATMENT (DIFFERENCES FROM ORAL TREATMENT)

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 410, 411	EEC (1983/84)	UK/HSC (1982) Test 3b Additional test 2b	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982) § 82-2 § 82-3	Japan/MAFF (1985)
ANIMALS						
1. Species/strain	adult rat, rabbit or guinea pig	=	=	=	=	adult rat, rabbit or guinea pig, etc. (mammals)
2. Weight at start	rats: 200 - 300 g; rabbits: 2 - 3 kg; guinea pigs: 350 - 450 g	not mentioned	not mentioned	=	=	=
3. Size of groups	at least 10M/10F (5M/5F for 21/28 d studies)	at least 10M/10F (5M/5F for 28 d studies)	at least 10M/10F (5M/5F for 28 d studies)	at least 10M/10F	at least 10M/10F (5M/5F for 21 d studies)	at least 5M/5F
4. Caging	individually	=	=	=	=	not mentioned
TREATMENT						
5. Administration	- concentration used should not damage skin	=	=	=	=	=
	- uniformly over an area approximately 10% of total body surface, or less if substance is very toxic. Treated area should be covered by film	=	=	=	=	=
	- limit test at 1000 mg/kg bw/d	=	=	=	=	=
7. Frequency of dosing	at least 6 h/d, 7 d/wk; 5 d/wk acceptable	at least 6 h/d, ideally 7 d/wk	at least 6 h/d, ideally 7 d/wk	=	=	=
8. Duration of treatment	21/28 d (410) or 90 d (411)	28 d (1984) or 90 d (1983)	28 d (3b) or 90 d (2b)	at least 90 d	21 d (§82-2) or 90 d (§ 82-3)	21 d
STUDY OBSERVATIONS						
10. Food consumption	weekly	=	=	=	=	= including calculation of food efficiency during the growing period
11. Water consumption	not mentioned	=	=	=	=	=
20. Histopathology	"organs as listed in continued 9", normal and treated skin	=	=	=	=	=

APPENDIX 2

(continued 6)

SUBCHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD-NOS. 412, 413

C. INHALATION EXPOSURE (DIFFERENCES FROM ORAL TREATMENT)

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 412, 413	EEC (1983/84)	UK/HSC (1982) Test 3c Additional test 2c	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982) § 82-4	Japan/MAFF (1985)
ANIMALS						
1. Species/strain	variety of species, but for rodents preferably the rat	rat	rat	variety of rodent species, rat preferred; if other mammalian species is used, justification	variety of rodent species, rat preferred; if other mammalian species is used, justification	at least one mammalian species, rat preferred
2. Age at start of dosing	young	=	=	young adult	young adult	young adult
Caging during exposure*	in groups by sex or individually	=	=	=	=	not mentioned
Equipment*	designed to sustain dynamic air flow of 12 - 15 air changes/h with 19% oxygen content and evenly distributed exposure atmosphere	designed to sustain dynamic air flow of at least 12 air changes/h with adequate oxygen content and evenly distributed exposure atmosphere	designed to sustain dynamic air flow of at least 12 air changes/h with 19% oxygen content and evenly distributed exposure atmosphere	=	=	inhalation equipment of appropriate performance should be used
	total "volume" of test-animals should not exceed 5% of test chamber volume.	=	=	=	=	
	oro-nasal or head-only exposure may be desirable	oro-nasal, head-only or whole-body individual eyposure may be used	oro-nasal, head-only or whole-body individual exposure may be used	=	=	
Physical measurements*	rate of air flow, continuously	=	=	rate of air flow continuously at least every 30 minutes	rate of air flow continuously at least every 60 minutes	rate of air flow
	actual test substance concentration should be held constant	actual test substance concentration in breathing zone (variation less than 15% of mean value, but wider range in some cases acceptable)	actual test substance concentration in breathing zone (variation less than ± 15% of mean value, but wider range in some cases acceptable)	actual test substance concentration in breathing zone	actual test substance concentration in breathing zone	actual concentrations of test substance
	analysis of constancy of particle size	=	=	=	=	particle size distribution
	temperature and humidity (preferably continously)	temperature and humidity	temperature and humidity	temperature and humidity at least every 30 minutes	temperature at least every 60 minutes	temperature and humidity
Diet*	no food during exposure time	=	=	=	=	=
Water*	no drinking water during exposure time	=	=	may be withheld in certain cases during exposure	should not come into direct contact with test atmospheres	should not come into direct contact with test atmospheres

* Additional items compared with tables for oral administration

APPENDIX 2

(continued 7)

SUBCHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD NO'S. 407, 408, 409

C. INHALATION EXPOSURE (DIFFERENCES FROM ORAL TREATMENT)

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983/84)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
TREATMENT						
5. Limit test	not mentioned	=	=	=	=	=
8. Frequency of exposure	6 h/d, 7 d/wk; 5 d/wk, acceptable	6 h/d; 5-7 d/wk	6 h/d (but other duration may be acceptable), 7 d/wk; 5 d/wk acceptable	=	6 h/d (but other duration may be acceptable), 7 d/wk; 5 d/wk acceptable	6 h/d, 5 d/wk
9. Duration of treatment	14/28 d (412) or 90 d (413)	28 d (1984) or 90 d (1983)	28 d (3c) or 90 d (2c)	at least 90 d	at least 90 d	90 d
CLINICAL DATA						
10. Food consumption	weekly	=	=	=	=	=
11. Water consumption	not mentioned	=	=	=	=	=
12. Clinical signs	as for oral treatment	=	=	=	=	observations before, during and after exposure
POST MORTEM EXAMINATIONS						
18. Gross pathology and organ weights	lungs should be removed intact, weighed and treated with a suitable fixative; nasopharyngeal tissue should be preserved in fixative	=	=	=	=	as for subchronic oral study
20. Histopathology	"organs as listed in continued 9", nasopharyngeal tissue in all animals of the high dose and control groups; organs showing gross lesions, target organs and lungs in all animals of all groups	"organs as listed in continued 9", respiratory tract/nasopharyngeal tissue in all animals of the high dose and control groups; organs showing gross lesions, target organs and lungs in all animals of all groups	"organs as listed in continued 9", respiratory tract/nasopharyngeal tissue in all animals of the high dose and control groups; organs showing gross lesions, target organs and lungs in all animals of all groups	=	=	as for subchronic oral study

APPENDIX 2

(continued 8)

SUBCHRONIC TOXICITY GUIDELINES

HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALYSIS PARAMETERS

Parameters in all guidelines	Additional parameters in individual guidelines	INDUSTRIAL CHEMICALS				PESTICIDES	
		OECD (1981)	EEC (1983/84)	UK/HSC (1982)	USA (EPA/ TSCA) (1983)	USA (EPA/ FIFRA) (1982)	Japan/MAFF (1985)
Haematology							
Clotting potential[1]							
Erythrocyte count (RBC)[a]							
Haemoglobin							
Haematocrit (PCV)[b]							
Total and differential WBC[c]							
Blood chemistry							
Albumin[3]	Alk. phosphatase						x
ALT (GPT)[3][d]	Bilirubin (total)	x	x[3]	x[3]	x	x	
AST (GOT)[3][e]	Calcium	x	x[4]	x[4]	x	x	
Glucose[4]	Chloride	x	x[4]	x[4]	x	x	
Total protein[3]	Creatinine	x	x[3]	x[3]	x	x	
Urea-N[3]	Gamma-glutamyl trans-peptidase	x	x	x	x		
	Ornithine decarboxylase	x	x	x	x		
	Phosphorus	x	x[4]	x[4]	x	x	
	Potassium	x	x[4]	x[4]	x	x	
	Sodium	x	x[4]	x[4]	x	x	
	Additional parameters	x[2]	x[2][4]	x[2][4]	x[2]	x[2]	
Urinalysis		x[5]	x[5][6]	x[5][6]	x[5]	x[5]	
	Appearance						x[7]
	Glucose						x[7]
	Ketones						x[7]
	Protein						x[7]
	Occult blood						x[7]
	Sediment						x[7]

1) such as prothrombin time, thromboplastin time and platelet count; Japan/MAFF: only platelet count mentioned
2) for adequate toxicological evaluation, or to extend the investigations of observed effects, additional analyses of parameters such as acid/base balance, cholinesterase act., hormones, lipids and methaemoglobin may be necessary
3) the only blood chemistry parameters required routinely in the 14/28-d studies (EEC, UK/HSC)
4) in 14/28-d studies (EEC, UK/HSC): only necessary for adequate toxicological evaluation or to extend the investigations of observed effects
5) not required on a routine basis, but only when there is an indication based on expected or observed toxicity
6) urinalysis not mentioned in 14/28-d studies (EEC; UK/HSC)
7) if considered necessary

a) RBC = red blood cell count
b) PCV = packed cell volume
c) WBC = white blood cell count
d) ALT = alanine aminotransferase
GPT = glutamic-pyruvic transaminase
e) AST = aspartate aminotransferase
GOT = glutamic-oxaloacetic transaminase

APPENDIX 2

(continued 9)

SUBCHRONIC TOXICITY GUIDELINES

ORGANS WEIGHED, PRESERVED AND EXAMINED HISTOPATHOLOGICALLY

Parameters in all guidelines	Additional parameters in individual guidelines	INDUSTRIAL CHEMICALS				PESTICIDES	
		OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/ TSCA) (1983)	USA (EPA/ FIFRA) (1982)	Japan/MAFF (1985)
Organ weights							
Kidneys	Adrenals	x	x	x	x		x
Liver	Brain				x[2]		
Testes	Lungs					x[3]	
Thyroids + parathyroids[1]	Ovaries				x		
Histopathology							
Target organs Gross lesions							
Adrenals[4]	Accessory genital organs	x[7]	x[7]	x[7]	x[9]	x[8]	x
Aorta	Bone marrow (sternum)	x[7]	x[7]	x[7]	x[7]	x[7]	x
Brain[10]	Bone (femur incl. joint)	x[6]	x[6]	x[6]	x[6][11]	x[6]	x
Caecum Colon Duodenum	Eyes	x[6]	x[6]	x[6]	x[6]	x[7]	x
Gonads*	Gall bladder (if present)	x	x	x	x[7]	x[7]	x
Heart[4]	Lacrimal glands	x[2][6]	x[2][6]	x[2][6]	x[2][6]	x[2][6]	
Ileum Jejunum	Nasopharyngeal tissue	x[3]	x[3]	x[3]	x[3]	x[3]	
Kidneys[4]	Respiratory tract		x[3]	x[3]			
Liver[4]	Salivary glands	x[7]	x[7]	x	x	x	x
Lungs	Skin	x[5][6]	x[5][6]	x[5][6]	x[5][6]	x[5][6][8]	x
Lymphnode(s)	Trachea	x[7]	x[7]	x[7]	x	x[7]	x
Mammary gland (females)[6]							
Musculature (thigh)[6]							
Nerve (peripheral)							
Oesophagus							
Pancreas							
Parathyroid(s)[7]							
Pituitary							
Rectum Spinal cord[6][11][13]							
Spleen[4]							
Stomach							
Thymus/thymic tissue							
Thyroid(s)							
Urinary bladder							
Uterus[12]							

* EPA/FIFRA - testes only

1) non rodents only
2) rodents only
3) after inhalation exposure only
4) the only organs required routinely in 14/21/28-d studies. However adrenals, heart and spleen not required in 28-d dermal studies
5) dermal studies: normal and treated skin
6) only if indicated by signs of toxicity or target organ involvement; Japan/MAFF: histopathological assessment of these organs in any case
7) organs sometimes required routinely, sometimes only after signs of toxicity or target organ involvement (see specific TG's for details)
8) not mentioned in 90 d-oral study
9) epididymis, prostate and, if present, seminal vesicles
10) including sections of medulla/pons, cerebellar cortex, cerebral cortex
11) evidently as the result of a printing error the complete sentence in question was not reproduced in the TSCA 90-d inhalation TG. In this specific case, therefore, the text of the TSCA 90-d oral and dermal TG was used and reproduced here by analogy
12) not mentioned in the following 90-d dermal studies: UK/HSC, OECD
13) cervical. thoracic. lumbar

APPENDIX 3

CHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD TG NO. 452

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 452	EEC (1983)	UK/HSC (1982) no guideline	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982) § 83-1	Japan/MAFF (1985)
ANIMALS						
1. Species/strain - rodents	rat of commonly used strain	=		=	=	=
- non-rodents	not mandatory; dog or primate	not specified		required, dog preferred	required, dog preferred	required, dog preferred
2. Age at start of dosing - rodents	as soon as possible after weaning	=		= ideally before 6 wk and not older than 8 wk	= ideally before 6 wk and not older than 8 wk	= ideally before 6 wk and not older than 8 wk
- non-rodents	young healthy animals	=		4 - 6 m, not more than 9 m	4 - 6 m, not more than 9 m	4 - 6 m, not more than 9 m
3. Size of groups - rodents	at least 20M/20F; higher numbers if interim sacrifices are planned	=		=	=	=
- non-rodents	at least 4M/4F; higher numbers if interim sacrifices are planned	=		=	=	=
4. Satellite groups - rodents and non-rodents	not mentioned	=		=	=	interim sacrifice recommended for rodents
TREATMENT						
5. Dose levels	at least 3 + control	=		=	=	=
- low dose	no evidence of toxicity	=		=	=	doses to give dose relationship and no-observable effect level
- intermediate dose(s)	not specified	mid-range between high and low doses		minimal toxic effects	minimal toxic effects	
- high dose	some signs of toxicity without causing excessive lethality	=		some signs of toxicity without causing excessive lethality (rodents), or no fatalities (non-rodents)	some signs of toxicity without causing excessive lethality (rodents), or no fatalities (non-rodents)	high dose < 5% if given in diet
6. Route of administration	a) oral	a) =		a) =	a) =	a) in diet (in principle)
	b) dermal	b) not mentioned		b) =	b) =	b) not mentioned
	c) inhalation (c.f. App. 6)	c) =		c) =	c) =	c) not mentioned

APPENDIX 3

(continued 2)

CHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD TG NO. 452

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
7. Frequency of dosing - rodents and non-rodents	a) oral, ideally 7 d/wk; 5 d/wk acceptable	a) =		a) =	a) =	a) not specified
	b) dermal, not specified	b) =		b) 6 h/d	b) 6 h/d	b) not mentioned
	c) inhalation, 6 h/d, 5 d/wk or 22 - 24 h/d, 7 d/wk (cf. Appendix 6)	c) =		c) 6 h/d	c) not specified	c) not mentioned
8. Duration of treatment - rodents	at least 12 m	=		=	at least 12 m for non-food use, 24 m for food use	24 m rats 18 m mice
- non-rodents	at least 12 m	=		=	=	=
STUDY OBSERVATIONS I CLINICAL DATA						
9. Body weights	weekly for first 13 wk then once every 4 wk	=		=	=	=
10. Food consumption	weekly for first 13 wk then at approx. 3-monthly intervals	=		weekly for first 13 wk then approx. monthly intervals (when test substance administered in diet)	weekly for first 13 wk then approx. monthly intervals	weekly for first 13 wk then once every 4 wk; includes calculation of food efficiency during the growing period of the test animals
11. Water consumption	not mentioned	=		weekly for first 13 wk then approx. monthly intervals (when test substance administered in drinking water)	weekly for first 13 wk then approx. monthly intervals	=
12. Clinical signs	observe at least once daily	=		=	=	=
13. Mortality	observe at least twice daily	=		=	=	observe at least once daily
14. Ophthalmology - rodents	not mentioned	=		at least 10 rats/ sex/group prior to start and at end of dosing. If changes are detected, then all animals	at least in high dose and control groups prior to start and at end of dosing. If changes are detected, then all animals	at least in high dose and control groups prior to start and at end of dosing. If changes are detected, then all animals
- non-rodents	not mentioned	=		prior to start and at termination on all animals	at least in high dose and control groups prior to start and at end of dosing. If changes are detected, then all animals	prior to start and at termination preferably on all animals

APPENDIX 3

(continued 3)

CHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD TG NO. 452

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
II HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALYSIS (cf. cont. 5)						
15. Haematology						
- rodents	10 rats/sex/group at 3 and 6 m and then approx. every 6 m and at termination	=		at least ... =	at least 10 rats/sex/group at approx. 6 m intervals and at termination (if haematological effects in the subchronic test: additional haematology after 3 m)	at least 10 rats/sex/group every 6 m and at termination (if haematological effects in the subchronic test: additional haematology after 3 m)
- non-rodents	all animals pretest, at 3 and 6 m and then approx. every 6 m and at termination	=		=	all animals at approx. 6 m intervals and at termination (if haematological effects in the subchronic test: additional haematology after 3 m)	all animals every 6 m and at termination (if haematological effects in the subchronic test: additional haematology after 3 m)
- rodents and non-rodents	differential blood counts on animals: - which show a deterioration in health - in the highest dose and control groups - in the lower dose group(s) if necessary	=		=	=	not specified
16. Clinical chemistry						
- rodents	10 rats/sex/group approx. every 6 m and at termination	=		at least 10 rats/sex/group at beginning, near middle and end of test period	at least 10 rats/sex/group at beginning, middle and end of test period	at least ... =
- non-rodents	all animals pretest and then approx. every 6 m and at termination	=		all animals at beginning, near middle and end of test period	all animals at beginning, middle and end of test period	all animals every 6 m and at termination
17. Urinalysis						
- rodents	10 rats/sex/group at 3 and 6 m and then approx. every 6 m and at termination	=		at least ... =	at least 10 rats/sex/group at approx. 6 m intervals and at termination	at least 10 rats/sex/group every 6 m and at termination
- non-rodents	all animals pretest, at 3 and 6 m and then approx. every 6 m and at termination	=		=	all animals at approx. 6 m intervals and at termination	all animals every 6 m and at termination

APPENDIX 3

(continued 4)

CHRONIC TOXICITY GUIDELINES / COMPARISON WITH OECD TG NO. 452

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
III POST MORTEM EXAMINATIONS (cf. cont. 6)						
18. Gross pathology - rodents	all animals	=		=	=	=
- non-rodents	all animals	=		=	=	=
19. Organ weights - rodents	10 rats/sex/group	=		at least 10 rodents/ sex/group	at least ... =	at least ... =
- non-rodents	all animals	=		=	=	=
20. Histopathology - rodents	a) all animals that died or were killed during study	a) =		a) =	a) =	a) =
	b) all animals in control and high dose groups	b) =		b) =	b) =	b) =
	c) animals from lower dose groups if necessary	c) =		c) =	c) =	c) =
	d) all animals: all gross lesions and target organs (including tumours)	d) =		d) = in addition: lungs, liver, kidneys	d) = in addition: lungs, liver, kidneys	d) = in addition: lungs, liver, kidneys
- non-rodents	a) all animals that died or were killed during study	a) =		all animals	all animals	all animals
	b) all animals in control and high dose groups	b) =				
	c) animals from lower dose groups if necessary	c) =				
	d) all animals: all gross lesions and target organs (including tumours)	d) =				

APPENDIX 3

(continued 5)

CHRONIC TOXICITY TEST GUIDELINES

HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALYSIS PARAMETERS

Parameters in all guidelines	Additional parameters in individual guidelines	INDUSTRIAL CHEMICALS				PESTICIDES	
		OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/ TSCA) (1983)	USA (EPA/ FIFRA) (1982)	Japan/MAFF (1985)
Haematology							
Clotting potential[1)]							
Erythrocyte count (RBC)[a)]							
Haemoglobin							
Haematocrit (PCV)[b)]							
Total and differential[2)] WBC[c)]							
Blood chemistry							
Albumin	Alk. phosphatase	x	x				x
ALT (GPT)[d)]	Bilirubin (total)				x	x	
AST (GOT)[e)]	Calcium				x	x	
Glucose	Chloride				x	x	
Total protein	Cholesterol				x	x	
Urea-N	Creatinine phosphokinase				x	x	
	Creatinine				x	x	
	Gamma-glutamyl transpeptidase	x	x		x		
	Ornithine decarboxylase	x	x		x		
	Phosphorus				x	x	
	Potassium				x	x	
	Sodium				x	x	
	Additional parameters				x[3)]	x[3)]	
Urinalysis							
Appearance	Bilirubin				x	x	
Glucose							
Ketones							
Occult blood							
Protein							
Sediment							
Specific gravity							
Volume							

1) such as prothrombin time, thromboplastin time and platelet count; Japan/MAFF: only platelet count mentioned

2) if necessary

3) for adequate toxicological evaluation or to extend the investigations of observed effects, additional analyses of parameters such as acid/base balance, cholinesterase act., hormones, lipids and methaemoglobin may be necessary

a) RBC = red blood cell count

b) PCV = packed cell volume

c) WBC = white blood cell count

d) ALT = alanine aminotransferase
GPT = glutamic-pyruvic transaminase

e) AST = aspartate aminotransferase
GOT = glutamic-oxaloacetic transaminase

APPENDIX 3

(continued 6)

CHRONIC TOXICITY TEST GUIDELINES

ORGANS WEIGHED, PRESERVED AND EXAMINED HISTOPATHOLOGICALLY

Parameters in all guidelines	Additional parameters in individual guidelines	INDUSTRIAL CHEMICALS				PESTICIDES	
		OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/ TSCA) (1983)	USA (EPA/ FIFRA) (1982)	Japan/MAFF (1985)
Organ weights							
Brain	Adrenals	x	x		x		x
Gonads*	Thyroids + parathyroids (non-rodents only)	x	x				x
Kidneys							
Liver	As many other organs as possible						x
Histopathology							
Target organs							
Gross changes							
Accessory genital organs[1]	Gall bladder[4]				x	x	x
Adrenals	Respiratory tract[5]	x	x		x	x	
Aorta							
Bone (femur incl. joint)							
Bone marrow (sternum)							
Brain[2]							
Caecum							
Colon							
Duodenum							
Eyes							
Gonads**							
Heart							
Ileum							
Jejunum							
Kidneys							
Liver							
Lungs							
Lymphnode(s)							
Mammary gland (females)							
Musculature							
Nerve (peripheral)							
Oesophagus							
Pancreas							
Parathyroid(s)							
Pituitary							
Rectum							
Salivary glands							
Skin							
Spinal cord[3]							
Spleen							
Stomach							
Thymus/thymic tissue							
Thyroid(s)							
Trachea							
Urinary bladder							
Uterus							

* EPA/FIFRA & Japan/MAFF - testes only

** EPA/FIFRA - testes only

1) EPA/TSCA: epididymis, prostate and, if present, seminal vesicles

2) including sections of medulla/pons, cerebellar cortex, cerebral cortex

3) cervical, thoracic, lumbar

4) if present

APPENDIX 4

CARCINOGENICITY TEST GUIDELINES / COMPARISON WITH OECD TG. NO. 451

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 451	EEC (1983)	UK/HSC (1982) Additional test 4	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982) § 83-2	Japan/MAFF (1985)
ANIMALS						
1. Species/strain - rodents	preferably rats and mice of strains with a low incidence of spontaneous tumours; good knowledge of tumour profile	preferably rat of commonly used laboratory strains	preferably rat of commonly used laboratory strains	preferably rats and mice of commonly used laboratory strains	preferably rats and mice of commonly used laboratory strains	preferably rats and mice of commonly used laboratory strains
- non-rodents	dog and primate mentioned, but no further specifications	mentioned, but no further specifications	not mentioned	not mentioned	not mentioned	not mentioned
2. Age at start of dosing	as soon as possible after weaning, preferably before 6 wk old	young healthy animals	young healthy animals	as soon as possible after weaning, ideally before 6 wk old but not older than 8 wk	as soon as possible after weaning, ideally before 6 wk old but not older than 8 wk	as soon as possible after weaning, ideally before 6 wk old but not older than 8 wk
3. Size of groups	at least 50M/50F; higher numbers if interim sacrifices are planned	=	=	=	=	=
4. Satellite groups	not mentioned	=	=	=	=	=
TREATMENT						
5. Dose levels	at least 3 + control	=	=	=	=	=
- low dose	no evidence of toxicity, not lower than 10% of high dose	=	=	=	not specified	not specified
- intermediate dose(s)	mid range between low and high dose	=	=	=	not specified	not specified
- high dose	evidence of minimal toxicity without substantially altering the normal life span due to effects other than tumours	=	=	evidence of minimal toxicity without substantially altering the normal life span	evidence of minimal toxicity without substantially altering the normal life span	=
	e.g. less than 10% depression of body weight gain	=	=	not mentioned	not mentioned	not mentioned
6. Route of administration	a) oral	a) =	a) =	a) =	a) =	a) in diet (in principle)
	b) dermal: not specified	b) =	b) =	b) topical application on approx. 10% of body surface, 6 h/d	b) topical application on approx. 10% of body surface, 6 h/d	b) not mentioned
	c) inhalation (c.f. App. 6)	c) =	c) =	c) =	c) =	c) not mentioned

APPENDIX 4

(continued 2)

CARCINOGENICITY TEST GUIDELINES / COMPARISON WITH OECD TG. NO. 451

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
7. Frequency of dosing	a) oral: ideally 7 d/wk; 5 d/wk acceptable	a) =	a) =	a) continously when administered in diet or water	a) =	a) not specified
	b) dermal: not specified	b) =	b) =	b) 6 h/d	b) 6 h/d	b) not mentioned
	c) inhalation: 6 h/d, 5 d/wk or 22-24 h/d, 7 d/wk	c) =	c) =	c) 6 h/d	c) not specified	c) not mentioned
8. Duration of treatment - rats	at least 24 m*	=	=	=	=	=
- mice (and hamsters)	at least 18 m*	=	=	=	=	=
STUDY OBSERVATIONS I CLINICAL DATA						
9. Body weights	weekly for first 13 wk then once every 4 wk	=	=	=	=	=
10. Food consumption	weekly for first 13 wk then at approx. 3 monthly intervals	=	=	weekly for first 13 wk then approx. monthly intervals (only when test substance is administered in diet)	weekly for first 13 wk then approx. monthly intervals	weekly for first 13 wk then once every 4 wk; includes calculation of feed efficiency during the growing period of the test animals
11. Water consumption	not mentioned	weekly for first 13 wk then at approx. 3-monthly intervals (only when test substance is administered in drinking water)	weekly for first 13 wk then at approx. 3-monthly intervals (only when test substance is administered in drinking water)	weekly for first 13 wk then approx. monthly (only when test substance is administered in drinking water)	weekly for first 13 wk then approx. monthly intervals	=
12. Clinical signs	observe at least once daily	=	=	=	=	=
13. Mortality	observe at least twice daily	observe regularly	observe regularly	=	=	observe at least once daily
14. Ophthalmology	not mentioned	=	=	=	=	=

* For strains of greater longevity or low spontaneous tumour rates other termination criteria apply.

APPENDIX 4

(continued 3)

CARCINOGENICITY TEST GUIDELINES / COMPARISON WITH OECD TG. NO. 451

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
II HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALYSIS						
15. Haematology	- blood smear from all animals at 12 and 18 m and prior to sacrifice	=	=	=	blood smear from 10 animals/sex/group at 12 and 18 m and at sacrifice	=
	- differential blood counts on animals: - which show a deterioration in health	=	=	=	=	not mentioned
	- in the highest dose and control groups	=	=	=	=	=
	- in the lower dose group(s) if necessary	=	=	=	=	=
16. Clinical chemistry	not mentioned	=	=	=	=	=
17. Urinalysis	not mentioned	=	=	=	=	=
III POST MORTEM EXAMINATIONS (cf. cont. 4)						
18. Gross pathology	all animals	=	=	=	=	=
19. Organ weights	not mentioned	=	=	=	10 rodents/sex/group	10 animals/sex/group
20. Histopathology	a) all animals that died or were killed during study	a) =	a) =	a) =	a) =	a) =
	b) all animals in control and high dose groups	b) =	b) =	b) =	b) =	b) =
	c) animals in lower dose groups if necessary	c) =	c) =	c) =	c) =	c) =
	d) all animals: all gross lesions and target organs (including tumours or lesions suspected of being tumours)	d) =	d) =	d) =	d) = in addition: lungs, liver, kidneys	d) = in addition: lungs, liver, kidneys

APPENDIX 4

(continued 4)

CARCINOGENICITY TEST GUIDELINES

ORGANS WEIGHED, PRESERVED AND EXAMINED HISTOPATHOLOGICALLY

Parameters in all guidelines	Additional parameters in individual guidelines	INDUSTRIAL CHEMICALS				PESTICIDES	
		OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
Organ weights	Adrenals						x
	Brain					x	x
	Kidneys					x	x
	Liver					x	x
	Testes					x	x
	As many other organs as possible						x
Histopathology							
Target organs Gross lesions/tumours							
Accessory genital organs[1]	Aorta		x	x		x	x
Adrenals	Gall bladder[4]						x
Bone (femur incl. joint)	Musculature	x			x	x	x
Bone marrow (sternum)	Respiratory tract[5]	x	x	x	x	x	
Brain[2]	Thigh musculature		x	x			
Caecum							
Colon							
Duodenum							
Eyes							
Gonads*							
Heart							
Ileum							
Jejunum							
Kidneys							
Liver							
Lungs							
Lymphnode(s)							
Mammary gland (females)							
Nerve (peripheral)							
Oesophagus							
Pancreas							
Parathyroid(s)							
Pituitary							
Rectum							
Salivary glands							
Skin							
Spinal cord[3]							
Spleen							
Stomach							
Thymus/thymic tissue							
Thyroid(s)							
Trachea							
Urinary bladder							
Uterus							

* EPA/FIFRA testes only

1) EPA/TSCA: epididymis, prostate and, if present, seminal vesicles
2) including sections of medulla/pons, cerebellar cortex, cerebral cortex
3) cervical, thoracic, lumbar
4) if present
5) in inhalation studies only: the entire respiratory tract including nose (nasal cavity), pharynx, and larynx

APPENDIX 5

COMBINED CHRONIC TOXICITY/CARCINOGENICITY GUIDELINES/ COMPARISON WITH OECD TG NO. 453

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 453	EEC (1983)	UK/HSC (1982) no guideline	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982) § 83-5	Japan/MAFF (1985)
ANIMALS						
1. Species/strain	typically rat	preferably rat of commonly used strain		preferably rat of commonly used strain	preferably rat of commonly used strain	preferably rat of commonly used strain
2. Age at start of dosing	as soon as possible after weaning, preferably before 6 wk old	as soon as possible after weaning		as soon as possible after weaning, ideally before the rats are 6 wk but not older than 8 wk	as soon as possible after weaning, ideally before the rats are 6 wk old but not older than 8 wk	as soon as possible after weaning, but in any case not more than 8 wk old
3. Size of groups	at least 50M/50F; higher numbers if interim sacrifices are planned	=		=	=	=
4. Satellite groups	satellite control group: 10M/10F; satellite dose group: 20M/20F	=		satellite control group and satellite dose group(s): at least 20M/20F	satellite control group: 10M/10F; satellite dose group(s): not exactly specified	satellite control group and satellite dose groups: at least 20M/20F
TREATMENT						
5. Dose levels a) For carcinogenicity testing:	at least 3 + control	=		=	=	=
- low dose	no evidence of toxicity	=		=	=	not specified
	not lower than 10% of the high dose	=		low incidence of fatalities	low incidence of fatalities	not specified
- intermediate dose(s)	mid range between the high and low dose	=		minimal observable toxic effects (low incidence of fatalities)	minimal observable toxic effects (low incidence of fatalities)	not specified
- high dose	evidence of **minimal** toxicity without substantially altering the normal life span due to effects other than tumours	=		signs of toxicity without substantially altering the normal life span due to effects other than tumours	signs of toxicity without substantially altering the normal life span due to effects other than tumours	=
b) For chronic toxicity testing:	an additional treated, and a concurrent control satellite group	additional treated satellite groups and a concurrent control satellite group		at least one additional treated and a concurrent control satellite group	at least one additional treated and a concurrent control satellite group	an additional treated satellite group and a concurrent control satellite group
	the additional, highest dose in satellite group should produce frank toxicity	the additional, highest dose in satellite group should produce definite signs of toxicity		=	=	=

APPENDIX 5

(continued 2)

COMBINED CHRONIC TOXICITY/CARCINOGENICITY GUIDELINES/ COMPARISON WITH OECD TG NO. 453

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
6. Route of administration	a) oral	a) =		a) =	a) = (preferred)	a) in diet (in principle)
	b) dermal	b) =		b) =	b) =	b) not mentioned
	c) inhalation (c.f. App. 6)	c) =		c) =	c) =	c) not mentioned
7. Frequency of dosing	a) **oral studies:** ideally 7 d/wk; 5 d/wk acceptable	a) =		a) continously if administered in diet or water	a) ideally 7 d/wk (kind of exposure not exactly specified)	a) not specified
	b) **dermal studies:** not specified	b) =		b) ideally for at least 6 h/d	b) ideally 7 d/wk (kind of exposure not exactly specified)	b) not mentioned
	c) **inhalation studies:** 6 h/d, 5 d/wk or 22 - 24 h/d, 7 d/wk	c) =		c) 6 h/d	c) ideally 7 d/wk (kind of exposure not exactly specified)	c) not mentioned
8. Duration of treatment						
a) For carcinogenicity testing						
- rats	at least 24 m*	=		=	=	over most of the life span
- mice (and hamsters)	at least 18 m*	=		=	=	over most of the life span
b) For chronic toxicity testing	at least 12 m	=		=	at least 12 m for non-food use, 24 m for food use	24 m (rats) 18 m (mice)
STUDY OBSERVATIONS						
I CLINICAL DATA						
9. Body weights	weekly for first 13 wk, then once every 4 wk	=		=	=	=
10. Food consumption	weekly for first 13 wk then at approx. 3-monthly intervals	=		weekly for first 13 wk then approx. monthly intervals (only when test substance is administered in diet)	weekly for first 13 wk then approx. monthly intervals	weekly for first 13 wk then once every 4 wk; includes calculation of food efficiency during the growing period of the test animals
11. Water consumption	not mentioned	weekly for first 13 wk then at approx. 3 monthly intervals thereafter (only when test substance is administered in drinking water)		weekly for first 13 wk then at approx. monthly intervals thereafter (only when test substance is administered in drinking water)	weekly for first 13 wk then approx. monthly intervals	=

* For strains of greater longevity or low spontaneous tumour rates other termination criteria apply

APPENDIX 5

(continued 3)

COMBINED CHRONIC TOXICITY/CARCINOGENICITY GUIDELINES/
COMPARISON WITH OECD TG NO. 453

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
12. Clinical signs	at least once daily	=		=	=	=
13. Mortality	observe at least twice daily	observe regularly		=	=	observe at least once daily
14. Ophthalmology	not mentioned	=		on at least 10 animals/sex/group prior to start and at end of dosing. If changes are detected, then all animals	prior to start and at end of dosing, preferably in all animals but at least in high dose and control animals. If changes are detected, then all animals	prior to start and at end of dosing, preferably in all animals but at least in high dose and control animals. If changes are detected, then all animals
II HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALYSIS (cf. cont. 5)						
15. Haematology	20 rats/sex/group	10 rats/sex/group		at least 20 rodents/sex/group	at least 10 rodents/sex/group	at least 10 animals/sex/group
	3 m, 6 m and at approx. 6 m intervals thereafter, and at termination	=		=	at approx. 6 m intervals and at termination	every 6 m and at termination; if haematological effects in the subchronic test: additional after 3 m
	differential blood counts on animals: - which show a deterioration in health - in the highest dose and control groups (same intervals as above) - in the lower dose group(s) if necessary	differential blood counts on animals: - which show a deterioration in health - in the highest dose and control groups - in the lower dose group(s) if necessary		differential blood counts on animals: - which show a deterioration in health - in the highest dose and control groups - in the lower dose group(s) if necessary	=	differential blood counts on animals: - sacrificed in the midcourse of the test
16. Clinical chemistry	10 rats/sex/group	=		at least 10 rodents/sex/group	at least 10 rodents/sex/group	at least 10 rodents/sex/group
	at approx. 6 m intervals and at termination	=		just prior to initiation of dosing, near the middle and at the end of the test period	=	=
17. Urinalysis	10 rats/sex/group	=		at least 10 rodents/sex/group	at least 10 rodents/sex/group	at least 10 animals/sex/group
	3 m, 6 m, and at approx. 6 m intervals, and at termination	=		=	at approx. 6 m intervals and at termination	at approx. 6 m intervals and at termination; if effects in the subchronic test: additional after 3 m

APPENDIX 5

(continued 4)

COMBINED CHRONIC TOXICITY/CARCINOGENICITY GUIDELINES/ COMPARISON WITH OECD TG NO. 453

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
III POST MORTEM EXAMINATIONS (cf. cont. 6)						
18. Gross pathology	all animals	=		=	=	=
19. Organ weights	10 animals/sex/group	=		at least 10 rodents/ sex/group	at least 10 rodents/ sex/group	all animals (main groups) and at least 10 animals/sex (satellite groups)
20. Histopathology	a) all animals that died or were killed during the study	a) =		a) =	a) =	a) =
	b) all animals of the highest dose group(s) and controls	b) =		b) =*)	b) =	b) =
	c) animals in lower dose groups if necessary	c) =		c) =	c) =	c) =
	d) all animals: all gross lesions and target organs (including tumours or lesions suspected of being tumours)	d) =		d) = in addition: lungs, liver and kidneys	d) = in addition: lungs, liver and kidneys	d) = in addition: lungs, liver and kidneys

*) Evidently as the result of a printing error the complete sentence in question was not reproduced. In this specific case, therefore, the text of the TSCA-chronic and carcinogenicity guidelines was used and reproduced here by analogy.

APPENDIX 5

(continued 5)

COMBINED CHRONIC TOXICITY / CARCINOGENICITY GUIDELINES

HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALYSIS PARAMETERS

Parameters in all guidelines	Additional parameters for individual guidelines	INDUSTRIAL CHEMICALS				PESTICIDES	
		OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/ TSCA) (1983)	USA (EPA/ FIFRA) (1982)	Japan/MAFF (1985)
Haematology							
Clotting potential[1]							
Erythrocyte count (RBC)[a]							
Haemoglobin							
Haematocrit (PCV)[b]							
Total and differential WBC[2][c]							
Blood chemistry							
Albumin							
ALT (GPT)[d]							
AST (GOT)[e]	Alk. phosphatase	x	x				x
Glucose	Bilirubin (total)				x	x	x
Total protein	Calcium				x	x	
Urea-N	Chloride				x	x	
	Cholesterol				x (total)	x (total)	
	Creatinine phosphokinase				x	x	
	Gamma-glutamyl transpeptidase	x	x		x		
	Ornithine decarboxylase	x	x		x		
	Phosphorus				x	x	
	Potassium				x	x	
	Sodium				x	x	
	Additional parameters				x[3]	x[3]	
Urinalysis							
Appearance	Bilirubin				x		
Glucose							
Ketones							
Occult blood							
Protein							
Sediment							
Specific gravity							
Volume							

1) such as prothrombin time, thromboplastin time and platelet count; Japan/MAFF: only platelet count mentioned

2) if necessary

3) for adequate toxicological evaluation or to extend the investigations of observed effects, additional analyses of parameters such as acid/base balance, cholinesterase act., hormones, lipids and methaemoglobin may be necessary

a) RBC = red blood cell count

b) PCV = packed cell volume

c) WBC = white blood cell count

d) ALT = alanine aminotransferase
GPT = glutamic-pyruvic transaminase

e) AST = aspartate aminotransferase
GOT = glutamic-oxaloacetic transaminase

APPENDIX 5

(continued 6)

COMBINED CHRONIC TOXICITY / CARCINOGENICITY GUIDELINES

ORGANS WEIGHED, PRESERVED AND EXAMINED HISTOPATHOLOGICALLY

Parameters in all guidelines	Additional parameters for individual guidelines	INDUSTRIAL CHEMICALS				PESTICIDES	
		OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
Organ weights							
Brain	Adrenals	x	x		x		x
Gonads*							
Kidneys	As many other organs as possible						x
Liver							
Histopathology							
Target organs							
Gross changes							
Accessory genital organs[1]	Aorta		x		x	x	x
Adrenals	Gall bladder[4]				x	x	x
Bone (femur incl. joint)	Respiratory tract[5]	x	x		x	x	
Bone marrow (sternum)	Uterus	x			x	x	x
Brain[2]							
Caecum							
Colon							
Duodenum							
Eyes							
Gonads**							
Heart							
Ileum							
Jejunum							
Kidneys							
Liver							
Lungs							
Lymphnode(s)							
Mammary gland (females)							
Musculature							
Nerve (peripheral)							
Oesophagus							
Pancreas							
Parathyroid(s)							
Pituitary							
Rectum							
Salivary glands							
Skin							
Spinal cord[3]							
Spleen							
Stomach							
Thymus/thymic tissue							
Thyroid(s)							
Trachea							
Urinary bladder							

* EPA/FIFRA and Japan/MAFF: testes only

** EPA/FIFRA testes only

1) EPA/TSCA: epididymis, prostate and, if present, seminal vesicles

2) including sections of medulla/pons, cerebellar cortex, cerebral cortex

3) cervical, thoracic, lumbar

4) if present

5) in inhalation studies only: the entire respiratory tract including nose (nasal cavity), pharynx, and larynx

APPENDIX 6

CHRONIC, CARCINOGENICITY AND COMBINED STUDIES / COMPARISONS WITH OECD NOS: 451, 452, 453

INHALATION EXPOSURE (DIFFERENCES FROM ORAL TREATMENT)

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 451, 452, 453	EEC (1983)	UK/HSC* (1982)	USA (EPA/TSCA) (1983)	USA (EPA/FIFRA) (1982) § 83-1, 83-2, 83-3	Japan/MAFF (1985)
Frequency of exposure - Occupational exposure simulation	6 h/d, 5 d/wk	=	=	6 h/d	not mentioned	not mentioned
- Environmental exposure simulation	22 - 24 h/d, 7 d/wk	=	=	not mentioned	not mentioned	not mentioned
- Air flow rate	12 to 15 air changes/h	at least 12 air changes/h	=	=	not mentioned	not mentioned
- Chambers	- control and exposure chambers identical	=	=	not mentioned	not mentioned	not mentioned
	- slight negative pressure in the chambers	=	=	=	=	not mentioned
- Physical measurements	- rate of air flow, continuously	=	=	=	=	not mentioned
	- concentration of test substance	=	- (variance ± 15%)	- "dynamic inhalation system" with analytical concentration control system	- "dynamic inhalation system" with analytical concentration control system	not mentioned
	- temperature: 22 ± 2°C	=	=	=	=	not mentioned
	- rel. hum.: 30 - 70%	=	=	- rel. hum: 40 - 60%	- rel. hum. 40 - 60%	not mentioned
	- particle size in case of aerosols	=	=	=	=	not mentioned
Number of control groups	negative control vehicle control	=	=	=	=	not mentioned

* only in the carcinogenicity TG

APPENDIX 7

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 414

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981) TG 414	EEC (1983) -	UK/HSC (1982) Additional Test 1 (b)	USA (EPA/TSCA) (1984) HC-Organ/ Tissue-Dev. Tox.	USA (EPA/FIFRA) (1982) § 83-3	Japan/MAFF (1985)
ANIMALS						
1. Species/strain	- commonly used laboratory strains; should not have low fecundity and should be characterized for response to teratogens	=	=	= ... for its sensitivity to developmental toxins - at least 2 mammalian species	= - at least 2 mammalian species	= - at least 2 mammalian species
- rodents	- rat preferred; mouse or hamster	=	=	- rat, mouse or hamster	- rat preferred	- rat preferred
- non-rodents	- rabbit preferred	=	=	- rabbit	=	=
2. a) Age at start of study	- healthy young adult virgin F of comparable age and size	=	=	- young adult animals (nulliparous F)	- young adult pregnant animals	- young adult of first pregnancy
	- acclimatised to laboratory conditions for at least 5 d prior to the test	=	=	- not mentioned	- not mentioned	- not mentioned
b) - randomisation	- animals should be randomized and assigned to the treatment groups before mating	=	=	- not mentioned	- not mentioned	- not mentioned
c) - mating	- naturally, with M of established fertility	=	=	- not specified	- naturally	- naturally
	- or by artificial insemination	=	=	- not specified	=	=
3. Size of groups	- adequate to ensure that sufficient pups are produced to permit an evaluation of teratogenic potential of the substance	= ... sufficient litters and pups ...	= ... sufficient litters and pups ...	= ... of the potential developmental toxicity	=	- not mentioned
- rodents	- at least 20 pregnant rats, mice or hamsters	=	=	=	=	=
- non-rodents	- at least 12 pregnant rabbits	=	=	=	=	=
4. Caging - rodents and non-rodents	- individual	=	=	- not mentioned	- not mentioned	- not mentioned
	- pregnant F may be provided with nesting materials	- not mentioned	- not mentioned	- not mentioned	- not mentioned	- not mentioned

APPENDIX 7

(continued 2)

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 414

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
TREATMENT						
5. Dose levels	- at least 3 + control + also vehicle control if the test substance is administered in a vehicle	=	=	= + concurrent control (untreated or sham-treated) and/or where appropriate a vehicle control if the test substance is administered in a vehicle	= + concurrent control (untreated or sham treated) or vehicle group if the test substance is administered in a vehicle of unknown toxicity	= + control or vehicle group if the test substance is administered in a vehicle
- low dose	- no observable effects	=	=	- no grosaly observable evidence of either maternal or developmental toxicity	- no evidence of toxicity	=
- intermediate dose(s)	- geometrically between high and low dose	=	=	- ideally, minimal observable toxic effects - if more than 1 intermediate concentration is used, the concentration levels should be spaced to produce a gradation of toxic effects	- not mentioned	=
- high dose	- ideally, some overt maternal toxicity, (e.g. slight weight loss, but not more than 10% maternal deaths) unless limited by the physical/chemical nature or biological properties of the substance	=	=	=	=	=
6. Limit test	- if a dose level of at least 1000 mg/kg produces no evidence of embryotoxicity or teratogenicity, studies at other dose levels may not be considered necessary	=	=	=	=	=
	- if a preliminary study at the high dose level, with definite evidence of maternal toxicity, shows no adverse effects on embryos, studies at other dose levels may not be considered necessary	- not mentioned	- if a dose less than 1000 mg/kg produces definite evidence of maternal toxicity, but shows no adverse effects on embryos, studies at other dose levels may not be considered necessary	- not mentioned	=	- not mentioned

APPENDIX 7

(continued 3)

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES/

COMPARISON WITH OECD TG NO. 414

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
7. a) Requirements for vehicle	- toxicological properties should be understood	=	=	- not mentioned	=	=
	- should not be teratogenic or have effects on reproduction	=	=	=	=	=
b) Requirements for control	- animals should be handled in an identical manner to the exposed animals	=	=	=	=	=
8. Exposure conditions	- test substance should be administered at approximately the same time each day	=	=	=	- not mentioned	=
	- when given by gavage, dose may be based on the body weight of the F at start of substance administration	- dose may be based on the body weight of females at start of substance administration	=	=	=	=
	- alternatively, the animals may be weighed periodically and the dosage based on the recent weight determination	=	=	=	=	=
9. Route of administration	- orally, by gavage	=	=	=	=	=
	- alternatively other routes may be used where these are more representative of likely routes of human exposure	= - or depending on the physical properties of the test substance	= - for example, by inhalation for volatile solvents	- unless the chemical or physical characteristics of the test substance, or pattern of human exposure suggest a more appropriate route of administration	- unless the chemical or physical characteristics of the test substance, or pattern of human exposure suggest a more appropriate route of administration	- not mentioned
10. Duration of treatment	- period of major organogenesis	=	=	=	=	=
	- alternatively, the period of dosing may be extended to approximately 1 d before expected delivery date	=	=	- not mentioned	=	=

APPENDIX 7

(continued 4)

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES/

COMPARISON WITH OECD TG NO. 414

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
- rodents	- rat: d of gest. 6 - 15	=	=	=	=	= or d 7 - 17
	- mouse: d of gest. 6 - 15	=	=	=	=	=
	- hamster: d of gest. 6 - 14	=	=	=	=	=
- non-rodents	- rabbit: d of gest. 6 - 18	=	=	=	=	=
- definition of day 0	- day on which vaginal plug and/or sperm are observed	=	=	=	=	=
	- if based on observation of mating or artificial insemination the times stated should be adjusted by adding 1 d	=	=	- not mentioned	=	=
11. Frequency of dosing	- daily	=	=	=	=	- not specified
STUDY OBSERVATIONS **I CLINICAL DATA**						
12. a) Body weight	- weekly	=	=	at least weekly	= ... and at the day of sacrifice	- period prior to, during and after treatment
b) Food consumption	- weekly	=	=	=	= ... in a dosed-feeding study	- period prior to, during and after treatment
13. Clinical examination a) Clinical signs/ Mortality	- at least once daily throughout the study	=	- during the treatment and observation period at least once each d	=	- at least once each week at the same time as weighing	=
	- daily additional observations to minimize loss of animals to the study	=	=	=	=	=
	- dead, weak or moribund animals should be removed and necropsied	- not mentioned	- not mentioned	=	= ... to ensure that not more than 10% of animals in any test group are lost due to cannibalism	- not specified

APPENDIX 7

(continued 5)

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 414

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1981)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
b) Abortion or premature delivery	- F should be sacrificed and subjected to thorough macroscopic examination	=	=	=	=	=
14. Date of sacrifice	- shortly before the expected date of delivery	=	=	=	=	- not specified
	- one day prior to term	=	=	- not mentioned	- not mentioned	- not mentioned
II POST MORTEM EXAMINATIONS						
15. Examination after caesarean section or death	- the uterus should be removed immediately	=	=	= ... and weighed	=	=
	- number of embryonic or foetal deaths and live foetuses	=	=	- embryonic or foetal deaths and number of viable foetuses	=	=
	- estimation of time of death in utero	=	=	=	=	=
	- number of corpora lutea in rats and rabbits may be determined	=	=	- number of corpora lutea should be determined for all species except mice	- number of corpora lutea where possible	- number of corpora lutea where necessary
16. Examination of foetuses	- externally	=	=	=	=	=
	- sex	=	=	=	=	=
	- individual weight	=	=	=	- litter weight	- litter weight
	- mean weight derived	=	=	=	=	=
17. Preparation and morphological examination of foetuses - rodents	- 1/3 to 1/2 of each litter, for skeletal anomalies	=	=	=	=	=
	- remaining part of each litter for soft tissue anomalies	=	= - including growth retardation, delayed ossification and haemorrhages	=	=	=
- non-rodents	- each foetus for visceral anomalies by dissection	=	=	=	=	=
	- then for skeletal anomalies	=	= - including growth retardation, delayed ossification and haemorrhages	=	=	=
18. Gross pathology	- examination for any structural abnormalities or pathological changes which may have influenced pregnancy	=	- any animals which die during the study ... =	=	=	=

APPENDIX 7

(continued 6)

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES

INHALATION EXPOSURE (DIFFERENCES FROM ORAL TREATMENT)

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD no guideline	EEC no guideline	UK/HSC no guideline	USA (EPA/TSCA) (1984) HG-Organ/Tissue Dev Tox-Inhal	USA (EPA/FIFRA) no guideline	Japan/MAFF no guideline
ANIMALS						
4. Caging				- individual		
TREATMENT						
5. Dose levels				- at least 3 + concurrent control (exposed to clean, filtered air) and where appropriate an additional vehicle control if the test substance under study requires a vehicle for delivery		
6. Limit test				- if a test at an exposure of 5 mg/l (actual concentration of inhalable substance), or (if this is not possible due to physical or chemical properties of the test substance) the maximum attainable dosis, produces no observable developmental toxicity, then a full study using 3 exposure levels might not be necessary		
7. Requirements for control				- identical conditions to the groups exposed to the substance		
8. Exposure conditions a) Inhalation equipment				- "dynamic inhalation system" with suitable concentration control/ monitoring system - same conditions throughout exposure chamber		

APPENDIX 7

(continued 7)

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES

INHALATION EXPOSURE (DIFFERENCES FROM ORAL TREATMENT)

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD	EEC	UK/HSC	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA)	Japan/MAFF
b) Physical measurements				- evenly distributed exposure atmosphere - 12 - 15 air changes/h - O_2-content 19% - Temperature rodents 22° ± 2°C non-rodents 20° ± 3°C - Rel. hum. 40 - 60%; but in certain instances (e.g. tests of aerosols, use of water vehicle) may not be practicable - Volume of test animals should not exceed 5% of test chamber		
				- Continuous monitoring and recording, at least every 30 minutes of air flow, temperature, rel. hum. - Continuous monitoring and recording, at least at beginning, intermediate time and end of exposure period, of actual concentration of test substance - particle size analysis		
9. Route of administration				- whole-body exposure - oro-nasal or head-only exposure requires justification/reasoning		
11. Frequency of exposure				- at least 6 h/d + appropriate additional time for chamber equilibrium		

APPENDIX 7

(continued 8)

REPRODUCTIVE TOXICITY GUIDELINES

TERATOGENICITY TEST GUIDELINES

INHALATION EXPOSURE (DIFFERENCES FROM ORAL TREATMENT)

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD	EEC	UK/HSC	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA)	Japan/MAFF
STUDY OBSERVATIONS						
I CLINICAL DATA						
12. b) Food consumption				- weekly - during exposure withdrawal of food; optional, withdrawal of water		
13. Clinical examination a) Clinical signs/ mortality				- particular attention to observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma		

APPENDIX 8

REPRODUCTIVE TOXICITY GUIDELINES

ONE-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 415

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983) TG 415	EEC (1983) -	UK/HSC (1982) Fertility study Additional test 1 (a)	USA (EPA/TSCA) no guideline	USA (EPA/FIFRA) no guideline	Japan/MAFF no guideline
ANIMALS						
1. Species/strain	- rat or mouse	=	- rat			
	- strains with low fecundity should not be used	=	- not mentioned			
2. Age at start of study						
- males	- 5 to 9 wk old, after weaning	=	- young, mature			
	- after at least 5 d of acclimatisation	=	- not mentioned			
- females	- young adult	- weaned	=			
	- after at least 5 d of acclimatisation	=	- not mentioned			
3. Size of groups	- sufficient number to yield about 20 pregnant F at or near term	=	- sufficient number to yield about 20 litters			
4. Caging	- pregnant F individually	- near parturition, pregnant F separately	- not mentioned			
	- provided with nesting materials	=	- not mentioned			
TREATMENT						
5. Dose levels	- at least 3 + control	=	=			
	- or vehicle control receiving the vehicle in the highest volume used	=	- not mentioned			
	- + paired-fed control if the test substance causes reduced dietary intake	=	- not mentioned			

APPENDIX 8

(continued 2)

REPRODUCTIVE TOXICITY GUIDELINES

ONE-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/

COMPARISON WITH OECD TG NO. 415

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA)	USA (EPA/FIFRA)	Japan/MAFF
- low dose	- ideally, no observable adverse effects on the parents or offspring	=	- not mentioned			
- intermediate dose(s)	- ideally minimal toxic effects	=	- not mentioned			
- high dose	- ideally, toxicity but no mortality in the parental animals, unless dose is limited by physical/chemical nature or biological effects	=	- some evidence of toxicity in the parent animals, e.g. reduction in weight gain			
6. Limit test	- if a dose of at least 1000 mg/kg produces no evidence of interference with reproductive performance, studies at other dose levels may not be considered necessary	=	- not mentioned			
	- if a preliminary study at the high dose level, with definite evidence of maternal toxicity, shows no adverse effects on fertility, studies at other dose levels may not be considered necessary	=	- not mentioned			
7. Requirements for the vehicle	- should be known not to produce toxic effects	=	- not mentioned			

APPENDIX 8

(continued 3)

REPRODUCTIVE TOXICITY GUIDELINES

ONE-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 415

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA)	USA (EPA/FIFRA)	Japan/MAFF
8. Exposure conditions	- when administered by gavage or capsule, dosage is based on the individual animal's body weight and adjusted weekly	=	- not mentioned			
	- for F during pregnancy, dose may be based on daily body weight or on body weight at d 0 or 6 of pregnancy, if desired	- for F during pregnancy, dosage may be based on the body weight at day 0 or 6 of the pregnancy, if desired	-not mentioned			
9. Route of administration	- in the diet or drinking water	=	- usually oral			
	- alternatively, other routes are acceptable	=	=			
10. Duration of treatment - males	- during growth and at least one complete spermatogenic cycle	=	- not mentioned			
	- in rats: for approx. 10 wk prior to the mating period	=	- throughout the breeding cycle			
	- in mice: for approx. 8 wk prior to and during the mating period	=	- not mentioned			
- females	- in rats and mice: for at least 2 complete oestrus cycles, for at least 2 wk prior to mating throughout the 3-week mating period, during pregnancy, and up to weaning of F_1 offspring	=	- in rats, at least 20 d prior to mating - throughout the breeding cycle			
11. Frequency of dosing	- 7 d/wk	=	- not mentioned			

APPENDIX 8

(continued 4)

REPRODUCTIVE TOXICITY GUIDELINES

ONE-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/

COMPARISON WITH OECD TG NO. 415

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA)	USA (EPA/FIFRA)	Japan/MAFF
Procedure						
12. Mating	- 1 : 1 (1 F is placed with the same M until pregnancy occurs or 3 wk have elapsed)	=	- not mentioned			
	- alternatively, 1 M : 2 F	=	- not mentioned			
- definition of day 0	- d on which vaginal plug or sperm are found	=	- not mentioned			
	- each morning the F should be examined for presence of sperm or vaginal plug	=	- not mentioned - where necessary, about 10 d later mating can be repeated and a 2nd litter bred			
13. Proof of fertility	- pairs that fail to mate should be evaluated to determine the cause of infertility, e.g. additional opportunities to mate with other proven sires or dams, examination of the oestrous cycle or spermatogenesis, microscopic examination of the reproductive organs	=	- not mentioned			
14. Rearing: Litter size - without standardisation	- dams are allowed to litter normally and rear their progeny to the stage of weaning	=	=			
- with standardisation	- on d 4 after birth selection of 4 M and 4 F per litter	- between d 1 and d 4 after birth .. =	- not mentioned			
	- elimination of runts only is not appropriate	- not mentioned	- not mentioned			
	- partial adjustment is accepted if the number of M and F pups prevents having 4 of each sex per litter; 5 M and 3 F also acceptable	=	- not mentioned			
	- adjustments are not applicable for litters of less than 8 pups	=	- not mentioned			

APPENDIX 8

(continued 5)

REPRODUCTIVE TOXICITY GUIDELINES

ONE-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/

COMPARISON WITH OECD TG NO. 415

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA)	USA (EPA/FIFRA)	Japan/MAFF
STUDY OBSERVATIONS						
I CLINICAL DATA						
15. a) Body weight	- P (M and F) on the first d of dosing, and weekly thereafter	=	- not mentioned			
b) Food consumption	- weekly during premating and mating periods	- daily during premating and mating periods	- not mentioned			
	- optionally, daily during pregnancy	- not mentioned	- not mentioned			
	- after parturition and during lactation, food consumption measurements on same day as the litters are weighed	=	- not mentioned			
16. Clinical examination	- at least once daily throughout the test period	=	- not mentioned			
	- record duration of gestation, signs of difficult or prolonged parturition	=	- not mentioned			
17. Examination of litters at birth	- as soon as possible after delivery	=	- not mentioned			
	- number of pups, stillbirths, live births	=	- not mentioned			
	- sex of pups	=	- not mentioned			
	- gross anomalies	=	- not mentioned			
18. Preservation of pups	- dead or moribund pups and pups sacrificed at d 4 should be studied for possible defects	=	- not mentioned			

APPENDIX 8

(continued 6)

REPRODUCTIVE TOXICITY GUIDELINES

ONE-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/

COMPARISON WITH OECD TG NO. 415

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA)	USA (EPA/FIFRA)	Japan/MAFF
19. Examination during lactation	- counting of live pups; - weighing of litters on the morning after birth, and at d 4, d 7 and weekly thereafter until termination of the study when animals should be weighed individually	= =	- observation of the post-natal development of each litter until weaning or preferably until sexual maturity			
	- physical or behavioural abnormalities in dams or offspring	=	- not mentioned			
20. Date of sacrifice - males	- at the end of the mating period	=	- not specified			
	- alternatively, may be retained on the diet for the possible production of a second litter and should be killed at the end of the study	=	- not mentioned			
- females	- not specified	- not specified	- not specified			
II POST MORTEM EXAMINATIONS						
21. Gross pathology	- at the time of sacrifice or death during the study, animals of the P-generation should be examined macroscopically for any structural abnormalities or pathological changes	=	- not specified			
	- special attention to the organs of reproductive system	=	- not specified			

APPENDIX 8

(continued 7)

REPRODUCTIVE TOXICITY GUIDELINES

ONE-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 415

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA)	USA (EPA/FIFRA)	Japan/MAFF
22. Preservation of organs	- of all animals if necessary	=	- not mentioned			
23. Histopathology	- if organs (cf. 24) have not been examined in other multiple dose studies	=	- on reproductive and other relevant tissues/organs where indicated			
	- all animals in control and high dose group	=	- not mentioned			
	- all animals dying during study (where practicable). Organs showing abnormalities in these animals should be examined in all other P-animals	=	- not mentioned			
	- microscopy of all tissues showing gross pathological changes	=	- not mentioned			
	- microscopy of reproductive organs of animals suspected of infertility	=	- not mentioned			
24. Organs to be investigated	- ovaries	=	- not mentioned			
	- uterus	=				
	- cervix	=				
	- vagina	=				
	- testes	=				
	- epididymes	=				
	- seminal vesicles	=				
	- prostata	=				
	- coagulating gland	=				
	- pituitary gland	=				
	- target organs	=				

APPENDIX 9

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983) TG 416	EEC (1983)	UK/HSC (1982) Additional Test 1 (c)	USA (EPA/TSCA) (1984) HG-Organ/ Tissue-Repro/Fert	USA (EPA/FIFRA) (1982) § 83-4	Japan/MAFF (1985)
		**	No guideline*			**
ANIMALS						
1. Species/strain	- rat or mouse	=		- rat preferred	=	- at least one mammalian species, rat preferred
	- strains with low fecundity should not be used	=		=	=	=
	- if other species are used appropriate modifications will be necessary	- not mentionod		- if another mammalian species is used, justification/reasoning for its selection to be given	- if another mammalian species is used, justification/reasoning for its selection to be given	- not mentioned
2. Age at start of study						
P-generation						
- males	- 5 to 9 wk old	=		- at an age of 8 wk	- about 8 wk	- not mentioned
	- after weaning, and acclimatisation for at least 5 d	=		- not mentioned	- not mentioned	- immediately after weaning, and acclimatisation for at least 1 wk
- females	- not mentioned	- after weaning		- at an age of 8 wk	- about 8 wk	- immediately after weaning, and acclimatisation for at least 1 wk
	- at least 5 d of acclimatisation	=		- not mentioned	- not mentioned	
				- nulliparous and nonpregnant	- nulliparous and nonpregnant	- nulliparous and nonpregnant
3. Size of groups	- sufficient number to yield about 20 pregnant F at or near term	=		- at least 20 M and sufficient number of F to yield at least 20 pregnant F at or near term	- at least 20 M and sufficient number of F to yield at least 20 pregnant F at or near term	- at least 20 M and sufficient number of F to yield at least 20 pregnant F at parturition
	- for substances that cause sterility this may not be possible	- not mentioned		- not mentioned	- not mentioned	- not mentioned
4. Caging	- pregnant F individually	- pregnant F near parturition, separately		- pregnant F near parturition, separately in delivery or maternity cages	- pregnant F near parturition, separately in delivery or maternity cages	- pregnant F near parturition, separately in delivery or maternity cages
	- provided with nesting materials	=		=	=	=

* In some circumstances 2- or 3-generation studies may be carried out as mentioned in the one-generation study

** Where necessary 3-generation studies may be conducted

APPENDIX 9

(continued 2)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
TREATMENT						
5. Dose levels	- at least 3 + control	=		=	=	=
	- or vehicle control receiving the vehicle in the highest volume used	=		=	=	=
	- + paired-fed control if the test substance causes reduced dietary intake	=		- not mentioned	- not mentioned	- not mentioned
				- in the low and intermediate dose groups and in the control group the incidence of fatalities should be low to permit a meaningful evaluation of the results	- in the low and intermediate dose groups and in the control group the incidence of fatalities should be low to permit a meaningful evaluation of the results	
- low dose	- ideally, no observable adverse effects on the parents or offspring	=		- no evidence of toxicity - where there is an "usable" estimation of human exposure the lowest dose should exceed this	- no evidence of toxicity - where there is an "usable" estimation of human exposure the lowest dose should exceed this	- no evidence of toxicity
- intermediate dose(s)	- ideally, minimal toxic effects	=		= - if more than one intermediate dose is used, the levels should be spaced to produce a gradation of toxic effects	= - if more than one intermediate dose is used, the levels should be spaced to produce a gradation of toxic effects	=
- high dose	- ideally, toxicity but no mortality in the parental (P) animals unless dose is limited by the physical/chemical nature or biological effects	=		- toxicity, but no mortality in the parental (P) animals - highest concentration should not exceed 5% in the diet	- toxicity, but no mortality in the parental (P) animals - highest concentration should not exceed 5% in the diet with the exception of nutrients	- toxicity, but no mortality in dams

APPENDIX 9

(continued 3)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/

COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
6. Limit test	- not mentioned	- in the case of substances of low toxicity, if a dose level of at least 1000 mg/kg bw produces no evidence of interference with reproductive performance, studies at other dose levels may not be considered necessary - if a preliminary study at the high dose level, with definite evidence of maternal toxicity, shows no adverse effect on fertility, studies at other dose levels may not be considered necessary		- not mentioned	- not mentioned	- not mentioned
7. a) Requirements for vehicle	- without toxic effects	"		- should not interfere with absorption of the test substance or produce toxic effects	- should not interfere with absorption of the test substance or produce toxic effects	- not mentioned
b) Requirements for control	- not mentioned	"		"	"	- treatment in a manner identical to the dosed group
8. Exposure conditions	- when administered by gavage or capsule, dose is based on the individual animals' body weight and is adjusted weekly	"		"	"	"
	- during pregnancy, dose is based on daily bodyweight or on bodyweight at d 0 or 6 of pregnancy, if desired	- during pregnancy, dose may be based on individual body weight at d 0 or 6 of pregnancy, if desired		- during pregnancy, dose may be based on body weight at d 0 and 6 of pregnancy	- during pregnancy, dose may be based on body weight at d 0 and 6 of pregnancy	- during pregnancy, dose may be based on individual body weight at d 0 and 6 of pregnancy

APPENDIX 9

(continued 4)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
9. Route of administration	- diet or drinking water	=		=	=	- diet in principle
	- other routes are acceptable	=		=	- alternatively gavage or capsules - oral route is preferred	- alternatively gavage or capsules
10. Duration of treatment P-generation (males) - rats and mice:	- during growth and at least one complete spermatogenic cycle	=		- not mentioned	- not mentioned	- not mentioned
- rats:	- for 10 wk prior to the mating period	=		- for at least 8 wk prior to and throughout the 3 week mating period	- for at least 8 wk prior to and throughout the mating period	- for at least 8 wk prior to and throughout the mating period, pregnancy, and up to the weaning of F_1-offspring
- mice:	- for 8 wk prior to the mating period	=		- not mentioned	- not mentioned	- not mentioned
P-generation (females)	- two complete oestrous cycles	=		- not mentioned	- not mentioned	- not mentioned
	- in rats and mice; for at least 2 wk prior to mating	=		- for at least 8 wk prior to the mating period	- for at least 8 wk prior to the mating period	- for at least 8 wk prior to the mating period
	- throughout the 3-wk mating period, pregnancy, and up to the weaning of the F_1-offspring	=		=	=	=
- F_1-generation selected for mating - males	- starts at weaning and ends with sacrifice	=		- starts after weaning, then throughout the mating period with the F_1-F (11 wk)	- starts after weaning, then throughout the mating period with the F_1-F (11 wk for mice, 17 wk for rats)	- from weaning, to weaning of F_2-offspring

APPENDIX 9

(continued 5)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
- females	- starts at weaning and ends with sacrifice	=		- starts after weaning, then throughout the mating period with the F_1-M (11 wk), pregnancy, and to the weaning of the F_2-offspring	- starts after weaning, then throughout the mating period with the F_1-M (11 wk for mice, 17 wk for rats) and to the weaning of the F_2-offspring	- from weaning, to weaning of F_2-offspring
F_2-generation	- not mentioned	=		=	=	- if necessary
11. Frequency of dosing	- 7 d/wk	=		- continuous exposure if given in diet or drinking water	- continuous exposure if given in diet or drinking water	- not mentioned
Procedure						
12. Mating - definition of day 0	- day on which vaginal plug or sperm are found (each morning the F should be examined for presence of sperm or vaginal plug)	=		=	=	=
- P-generation	- 1 : 1; 1 F is placed with the same M until pregnancy occurs or 3 wk have elapsed	=		- each F with a single M from the same dose level until pregnancy occurs or 3 wk have elapsed	- each F with a single randomly selected M from the same dose level until pregnancy occurs or 3 wk have elapsed	- 1 F with a single M from the same dose group until mating is confirmed or 3 wk have elapsed
	- alternatively 1 M: 2 F possible	=		- paired mating should be clearly identified - mixed matings with other M should be avoided - not mentioned	- paired mating should be clearly identified - mixed matings with other M should be avoided - not mentioned	- not mentioned
- F_1-generation	- in rats: begins at the age of at least 13 wk	=		- in rats: begins at the age of approx. 14 wk	- in rats: begins at the age of approx. 17 wk	- not mentioned
	- in mice: begins at the age of at least 11 wk	=		- not mentioned	=	- not mentioned
	- 1 M and 1 F are randomly selected from each litter for cross-mating with a pup of another litter of the same dose group	=		=	- 1 M and 1 F are randomly selected from each litter for cross mating with a pup of another litter - mating of siblings should be avoided	- 1 or 2 M and 1 or 2 F = - mating of siblings should be avoided

* This was calculated according to tables published in the respective TG's

APPENDIX 9

(continued 6)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
	- in certain instances such as poor reproductive performance in the controls, consideration should be given to the production of 2 litters per generation	- not mentioned		- not mentioned	- not mentioned	- not mentioned
13. Proof of fertility	- pairs that fail to mate should be evaluated to determine the cause of infertility	=		=	=	=
	- additional opportunities to mate with other proven sires or dams	=		=	=	=
	- examination of the oestrous cycle or spermatogenesis	=		=	- not mentioned	=
	- microscopic examination of the reproductive organs	=		=	=	=
14. Rearing F_1- and F_2-generation - litter size without standardisation:	- dams are allowed to litter normally and rear their progeny to the stage of weaning	=		- not mentioned	- not mentioned	- not mentioned
- litter size with standardisation:	- on d 4 after birth, selection of 4 M and 4 F per litter	- between day 1 and day 4 after birth, selection of 4 M and 4 F per litter, as nearly as possible		=	=	=
	- elimination of runts only is not appropriate	- not mentioned		=	- not mentioned	- not mentioned
	- partial adjustment is accepted if the number of M and F pups prevents having 4 of each sex per litter. 5 M and 3 F are also acceptable	=		=	=	=
	- adjustments are not applicable for litters of less than 8 pups	=		=	=	=

APPENDIX 9

(continued 7)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
STUDY OBSERVATIONS						
I CLINICAL DATA						
15. a) Body weight P-generation parental	- on day 1 of dosing and weekly thereafter	=		=	=	=
F_1-generation parental	- on day 1 of dosing and weekly thereafter	=		- at birth, and d 4, 7 (optional) 14, 21 after birth	- at birth, and d 4, 7 (optional) 14, 21 after birth	=
b) Food consumption	- weekly during premating and mating periods	=		- not mentioned	- not specified	- not specified
	- optionally, daily during pregnancy	=		- not mentioned	- not specified	- not specified
	- after parturition and during lactation on the same d as the litters are weighed	=		- not mentioned	- not specified	- not specified
16. Clinical examination	- at least once daily throughout the study period	=		=	=	=
	- record duration of gestation, signs of difficult or prolonged parturition	=		=	=	=
17. Examination of litters at birth	- as soon as possible after delivery	=		=	=	=
	- number of pups, stillbirths, live births	=		=	=	=
	- sex of pups	=		- not mentioned	- not mentioned	- not mentioned
	- gross anomalies	=		=	=	=

APPENDIX 9

(continued 8)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
18. Preservation of pups	- dead or moribund pups and pups sacrificed at d 4 should be preserved and studied for possible defects	=		- dead pups and pups sacrificed at d 4 should be preserved and studied for possible defects and cause of death	- dead pups and pups sacrificed at d 4 should be preserved and studied for possible defects and cause of death	=
19. Examination during lactation	- counting of live pups	=		=	=	=
	- weighing of litters on the	=		- weighing of individual pups	=	- weighing of individual pups
	- morning after birth	=		- at birth, or soon thereafter	- at birth, or soon thereafter	- at birth, or soon thereafter
	- d 4	=		=	=	=
	- d 7	=		=	= (optional)	= (optional)
	- weighing individually weekly thereafter until termination of the study	=		- d 14 d 21 after parturition	- d 14 (optional) d 21 after birth, individual weighing of pups	- d 14 d 21 after birth
	- physical or behavioural abnormalities in dams or offspring should be recorded	=		=	=	=
20. Dates of sacrifice P-generation	- at the end of the mating period	=		=	=	- after weaning of the F_1-offspring
- males	- alternatively, may be retained on diet for the possible production of a 2nd litter, sacrificed and examined at same time before the end of study	=		- not mentioned	- not mentioned	- not mentioned
	- when they are no longer necessary for assessment of reproductive effects	=		- not mentioned	- not mentioned - after delivery of the litter last sired - or in cases of infertility after proof of fertility	- not mentioned
- females	- when they are no longer necessary for assessment of reproductive effects	=		- after weaning of the F_1-offspring	- after weaning of their last litters - or in cases of infertility after proof of fertility	- after weaning of the F_1-offspring

APPENDIX 9

(continued 9)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
F_1-generation selected for mating - males	- when they are no longer necessary for assessment of reproductive effects	=		- at the end of the mating period	- after mating period - after delivery of the last F_1-litter sired - or in cases of infertility after proof of fertility	- after weaning of the F_2-offspring
- females	- when they are no longer necessary for assessment of reproductive effects	=		- after weaning of the F_2-offspring	- after weaning of their last litters	- after weaning of the F_2-offspring
- males, females not selected for mating	- after weaning	=		=	=	=
F_2-offspring	- after weaning	=		= (d 21 after birth)	- at age 21 d	=
II POST MORTEM EXAMINATIONS						
21. Gross pathology	- at the time of sacrifice or death during the study, all parental animals (P and F_1) should be examined macroscopically for any structural abnormalities or pathological changes	=		- all animals including those which died during the experiment or were sacrificed in moribund condition should be completely examined	- all animals including those which died during the experiment or were sacrificed in moribund condition should be completely examined to ensure that not more than 10% of the animals in any test group are lost due to cannibalism	- on all animals at sacrifice, dead, or sacrificed in moribund state
	- special attention to the organs of reproductive system	=		=	=	=

APPENDIX 9

(continued 10)

REPRODUCTIVE TOXICITY GUIDELINES

TWO-GENERATION REPRODUCTION TOXICITY TEST GUIDELINES/ COMPARISON WITH OECD TG NO. 416

	INDUSTRIAL CHEMICALS				PESTICIDES	
	OECD (1983)	EEC (1983)	UK/HSC (1982)	USA (EPA/TSCA) (1984)	USA (EPA/FIFRA) (1982)	Japan/MAFF (1985)
22. Preservation of organs	- all P- and F_1-animals selected for mating	=		=	=	- organs of the reproductive system of all animals - those which are prepared for future examination should be embedded in paraffin
23. Histopathology	- if necessary, or if organs (c.f. 24) have not been examined in other multiple-dose studies	=		- not mentioned	- not mentioned	- not mentioned
	a) all animals in control and high dose groups (P- and F_1-generation) selected for mating	=		=	=	=
	b) all animals dying during study (where practicable)	=		- not mentioned	- not mentioned	- not mentioned
	c) organs showing abnormalities in these animals should be examined in animals from the other dose groups	=		=	=	=
	d) microscopy of all tissues showing gross pathological changes	=		=	=	=
	e) microscopy of reproductive organs of animals suspected of infertility	=		- not mentioned	- not mentioned	- not mentioned
24. Organs to be investigated	- ovaries	=		=	=	=
	- uterus	=		=	=	=
	- cervix	=		=	=	- not mentioned
	- vagina	=		=	=	=
	- testes	=		=	=	=
	- epididymes	=		=	=	=
	- seminal vesicles	=		=	=	=
	- prostata	=		=	=	=
	- coagulating gland	=		=	=	- not mentioned
	- pituitary gland	=		=	=	=
	- target organs	=		=	=	- not mentioned

F. BIBLIOGRAPHY

- Cohen, A.J.(1982). Correlation between subacute toxicity (4 weeks) and subchronic toxicity (13 weeks). Commission of the European Communities, Industrial Health and Safety: Quality Assurance of Toxicological Data, by W.J. Hunter (EEC) and C. Morris (EPA). Report, EUR 7270, 47.
- ECETOC (1983). Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology). Monograph No.5.
- EEC (1983). Annex V, EEC Directive 79-831. Part B. Toxicological Methods of Annex VIII, Draft. V/E/2/LUX/40/83, Revision 1.
  Introduction.
  Subchronic oral toxicity test: 90 day repeated oral dose test using rodent species.
  Subchronic oral toxicity study : 90 day repeated oral dose test using non-rodent species.
  Subchronic dermal toxicity study : 90 day repeated dermal dose study using rodent species.
  Subchronic inhalation toxicity study : 90 day repeated inhalation dose study using rodent species.
  Teratogenicity study - rodent and non-rodent.
  Chronic toxicity test.
  Carcinogenicity test.
  Combined carcinogenicity and chronic toxicity test.
  One-generation reproduction toxicity test.
  Two-generation reproduction toxicity test.
- EEC (1984). Commission directive of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (84/449/EEC). Off. J., L251, 27, 1.
  Part B : Methods for the determination of toxicity.
  General introduction.
  B.7. Subacute toxicity (oral).
  B.8. Subacute toxicity (inhalation).
  B.9. Subacute toxicity (dermal).
- EPA/FIFRA (1982). Pesticide Assessment Guidelines, subdivision F, Hazard Evaluation: Human and Domestic Animals; Office of Pesticide Programs, Draft, EPA 540/9-82-025, US Environmental Protection Agency, Office of Pesticide and Toxic Substances, Washington D.C. 20460; PB 83-153916.
  Organisation and Philosophy of Subdivision F.
  § 82-1 Subchronic Oral Toxicity (Rodent and Non-rodent) : 90 Day Study.
  § 82-2 Repeated Dose Dermal Toxicity: 21 Day Study.
  § 82-3 Subchronic Dermal Toxicity : 90 Day Study.
  § 82-4 Subchronic Inhalation Toxicity : 90 Day Study.
  § 83-1 Chronic Toxicity Studies.
  § 83-2 Oncogenicity Study.
  § 83-3 Teratogenicity Study.
  § 83-4 Reproductive and Fertility Effects.
  § 83-5 Combined Chronic Toxicity/Oncogenicity Studies.

- EPA/TSCA (1982). US Environmental Protection Agency, Health Effects Test Guidelines; EPA 560/6-82-001, Washington D.C. 20460, PB 82-232984. Reproduction and Fertility Effects (HG-Organ/Tissue-Repro/Fert.).
- EPA/TSCA (1983-84). Health Effects Test Guidelines; US Environmental Protection Agency, Office of Pesticide and Toxic Substances, Washington D.C. 20460; PB 84-2332. Published Oct. 1984.
  Subchronic Exposure Dermal Toxicity (HG-Subchronic - Dermal) (1983).
  Subchronic Exposure Inhalation Toxicity (HG-Subchronic - Inhal.)(1983).
  Subchronic Exposure Oral Toxicity (HG-Subchronic - Oral)(1983).

Chronic Exposure/Chronic Toxicity (HG-Chronic)(1983).
Oncogenicity (HG-Chronic-Onco)(1983).
Combined Chronic Toxicity/Oncogenicity (HG-Chronic-Combined)(1983).
Developmental Toxicity Study (HG-Organ/Tissue-Dev.Tox.)(1984). (Formerly Teratogenicity Study, HG-Organ/Tissue-Terato, 1983).

- GIFAP (1982). Position paper: Chronic toxicity in the dog. GIFAP Bulletin, 8(4), 1.
- IARC (1980). IARC Monographs on Evaluation of Carcinogenic Risk of Chemicals to Humans. Supplement 2. Long-term and short-term screening assays for carcinogens : a critical appraisal.
- OECD - Guidelines for Testing of Chemicals, Section 4 : Health Effects.
  407 - Repeated Dose Oral Toxicity - Rodent : 28-day or 14-day Study: (Adopted 12 May 1981).
  408 - Subchronic Oral Toxicity - Rodent : 90-day Study (Adopted 12 May 1981).
  409 - Subchronic Oral Toxicity - Non-rodent : 90-day Study (Adopted 12 May 1981).
  410 - Repeated Dose Dermal Toxicity : 21/28-day Study (Adopted 12 May 1981).
  411 - Subchronic Dermal Toxicity - 90-day Study (Adopted 12 May 1981).
  412 - Repeated Dose Inhalation Toxicity : 28-day or 14-day Study (Adopted 12 May 1981).
  413 - Subchronic Inhalation Toxicity - 90-day Study (Adopted 12 May 1981).
  414 - Teratogenicity (Adopted 12 May 1981).
  415 - One-Generation Reproduction Toxicity Study (Adopted 26 May 1983).
  416 - Two-Generation Reproduction Toxicity Study (Adopted 26 May 1983).
  451 - Carcinogenicity Studies (Adopted 12 May 1981).
  452 - Chronic Toxicity Studies (Adopted 12 May 1981).
  453 - Combined Chronic Toxicity/Carcinogenicity Studies (Adopted 12 May 1981).
- Japan/MAFF (1985). Testing Guidelines for the Evaluation of Safety of Agricultural Chemicals. The Ministry of Agriculture, Forestry and Fisheries.
  - Basic Approaches for these Guidelines.
  - Subchronic Oral Toxicity Study.
  - Subacute Dermal Toxicity Study.
  - Subchronic Inhalation Toxicity Study.
  - Chronic Toxic Study.
  - Oncogenicity Study.
  - Chronic Toxicity/Oncogenicity Combined Study.
  - Reproduction Study.
  - Teratology Study.
  - How to Compile Toxicology Study Reports.

  (This draft was made official in Jan. 1985, but no official English translation of the official version was available when this Monograph was published).
- NTP (1984). Report of Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation of the National Toxicology Programme. Board of Scientific Counselors, August 17, 1984. US-DHSS, Public Health Services.
- OECD (1981-a). Organisation for Economic Co-operation and Development, Principles for Good Laboratory Practice.
- OECD (1981-b). Decision of the OECD Council concerning the mutual acceptance of data in the assessment of chemicals (1981; C(81) 30 Final).
- UK, Health and Safety Commission (HSC)(1977). Discussion document: Proposed Scheme for the Notification of the Toxic Properties of Substances. HMSO London, 1.
- UK, Health and Safety Commission (HSC)(1982). Methods for the Determination of Toxicity; Notification of New Substances Regulations.
  Approved Code of Practice.
  - Test methods.
  - Test 3: Investigation of subacute toxic effect.

- a) Subacute oral toxicity (28 day repeated oral dose toxicity study).
- b) Subacute dermal toxicity (28-day repeated dermal dose toxicity study).
- c) Subacute inhalation toxicity (28 day repeated dose inhalation toxicity study).
- Additional test methods.
- Additional Test 1, Investigation of effects on reproduction :
- a) Fertility.
- b) Teratogenicity.
- c) Further investigations of effects on reproduction.
- Additional Test 2, Investigation of subchronic /long term toxicity (excluding carcinogenicity):
- a) Subchronic 90-day repeated-dose oral study using rodent species.
- b) Subchronic 90-day repeated-dose dermal study.
- c) Subchronic 90-day repeated-dose inhalation study.
- d) Subchronic 90-day repeated-dose oral study using non-rodent species.
- Additional Test 4, Carcinogenicity studies.
- Additional Test 5, Acute and subacute toxicity studies in additional species.

G. MEMBERS OF THE TASK FORCE

C. Baeder	Pharma Toxicological Research, Hoechst, Frankfurt
J. Hellwig	Department of Toxicology, BASF, Ludwigshafen
M.H. Litchfield	Plant Protection Division, ICI, Haslemere
F.A. Meier (Chairman)	Protection of Health and Environment, Ciba-Geigy, Basel
W. Rozenboom	Health, Safety and Environment Division, Shell Internationale Petroleum Mij, Den Haag
R.G. van Versendaal	Toxicology Department, Duphar, Weesp

H. MEMBERS OF THE SCIENTIFIC COMMITTEE

I. PURCHASE (Chairman), Director, Central Toxicology Laboratory	ICI (Alderley Park)
M. SHARRATT, (Vice-chairman), Senior Toxicologist	BP (Sunbury)
J. BACKSTROM, Consultant Toxicologist	ASSOCIATION OF SWEDISH CHEMICAL INDUSTRIES (Stockholm)
B. BROECKER, Coordinator, Product-related Environmental Problems	HOECHST (Frankfurt)
L. CAILLARD, Industrial Toxicological Service	RHONE-POULENC (Paris)
H.O. ESSER, Head of Biochemistry, Agricultural Division	CIBA-GEIGY (Basel)
P. GELBKE, Head, Department of Toxicology	BASF (Ludwigshafen)
U. KORALLUS, Medical Director	BAYER (Leverkusen)
H.G. NOSLER, Head, Coord. Centre for Environmental Protection and Consumer Safety	HENKEL (Düsseldorf)
J.F. NEWMAN, Consultant to ICI	(Reading)
S. PAGLIALUNGA, Department of Medicine and Industrial Hygiene	MONTEDISON (Milano)
C.L.M. POELS, Envir. Affairs Division	SHELL (den Haag)
C. DE SLOOVER, Head of Medical Department	SOLVAY (Brussels)
W.F. TORDOIR, Head of Occupational Health and Toxicology Division	SHELL (den Haag)
H. VERSCHUUREN, Head, Department of Toxicology	DOW CHEMICAL (Horgen)